



## REVIEW

# Apoptosis in the vasculature: mechanisms and functional importance

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Apoptotic death has now been recognized in a number of common and threatening vascular diseases, including atherosclerosis. Interest in apoptosis research relates to the fact that apoptosis, in contrast to oncosis, is a highly regulated process of cell death which raises the hope for the development of specific therapeutic strategies to alter disease progression. This review summarizes the mechanisms involved in vascular endothelial and smooth muscle cell survival/apoptosis, and the potential roles of apoptotic death in atherosclerosis and restenosis. The potential effects of modulation of apoptosis in these diseases are also discussed.

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**Keywords:** Apoptosis; atherosclerosis; caspases; endothelial cell; macrophage; smooth muscle cell; thrombosis

**Abbreviations:** ACE: angiotensin-converting enzyme; AIF: apoptosis inducing factor; Akt/PKB: protein kinase B; Ang II: angiotensin II; APAF-1: apoptotic protease-activating factor 1; CAD: caspase-activated deoxyribonuclease; Caspases: cysteine aspartate-specific proteases; CDKI: cyclin-dependent kinase inhibitor; CIDE: cell death-inducing DFF45-like effector; CLA-1: CD36 and LIMPII analogous-1; DFF: DNA fragmentation factor; EC: endothelial cell; EGF: epidermal growth factor; FADD: Fas-associated death domain; FAK: focal adhesion kinase; FasL: Fas ligand; FGF-2: basic fibroblast growth factor; HUVEC: human umbilical vein endothelial cell; ICAD: inhibitor of caspase-activated deoxyribonuclease; IFN: interferon; IGF: insulin-like growth factor; IL: interleukin; LDL: low density lipoprotein; LOX-1: lectin-like oxLDL receptor-1; LPS: lipopolysaccharide; MMP: matrix metalloproteinases; NO: nitric oxide; oxLDL: oxidized low density lipoprotein; PARP: poly(ADP-ribose) polymerase; PBMC: peripheral blood mononuclear monocyte; PDGF: platelet-derived growth factor; PI3K: phosphoinositide 3 kinase; PS: phosphatidylserine; RIP: receptor-interacting protein; SAPK: stress-activated protein kinase; SOD: superoxide dismutase; SR: scavenger receptor; TF: tissue factor; TIMP-1: tissue inhibitor of metalloproteinases-1; TNF: tumour necrosis factor; TNFR1: tumour necrosis factor receptor 1; TRADD: tumour necrosis factor receptor-associated death domain; VEGF: vascular endothelial growth factor; VSMC: vascular smooth muscle cell; XIAP: X-linked inhibitor of apoptosis

## Introduction

Vascular pathologists have long been intrigued by the observation of cell death within the arterial wall in atherosclerosis (Thomas *et al.*, 1976; Virchow, 1858) or during normal aging (Cliff, 1970) in the absence of overt necrosis. Rational explanations for these observations may now be at hand after the description by Kerr and colleagues in the early 1970s of a novel form of cell death distinct from necrosis, which they designated ‘apoptosis’ (from the Greek word for ‘falling’) (Kerr *et al.*, 1972). It has since been increasingly recognized that apoptosis may be involved in a number of critical events occurring during normal development and may play a key role in a wide variety of diseases (Hetts, 1998).

In contrast to the cellular and organelle swelling, and to the blebbing and membrane rupture associated with the classic form of induced cell death or ‘oncosis’, apoptosis was initially described in cells that are programmed to die on the basis of characteristic morphology (Kerr *et al.*, 1972). Cells undergoing apoptosis show cell shrinkage, chromatin margination and condensation with subsequent internucleosomal fragmentation of DNA, membrane redistribution of phospholipids (Martin *et al.*, 1995) and budding (emission of pseudopodia), with maintenance of membrane integrity. The process tends to break off into apoptotic bodies that are recognized and rapidly

engulfed by professional macrophages or adjacent neighbouring cells without inducing an inflammatory response. However, cell corpses that escape phagocytosis and remain free undergo secondary necrosis (Majno & Joris, 1995).

## Molecular mechanisms of apoptosis

Apoptosis can be initiated by *intrinsic signals*, during normal development for example. This occurs when cells activate an internal program of self-destruction (cell suicide, programmed cell death) in response to an internal clock, withdrawal of survival factors, changes in haemodynamic parameters or loss of contact. Every cell already contains all the components of the suicide machinery and is ready to engage in self-destruction unless it is actively signalled not to do so.

On the other hand, apoptosis can be initiated by a wide variety of *extrinsic signals* such as cytokines, hormones, oxidized lipids, chemotherapeutic, ionizing or viral agents (Haunstetter & Izumo, 1998). In contrast to the more chronic and progressive loss of cells that occurs during normal development, apoptosis triggered by extrinsic signals is generally more acute and massive. Therefore, the capacity for removal of apoptotic cells may be overcome and secondary necrosis of unremoved apoptotic cells is frequent. This may lead to chronic accumulation of cellular debris with the potential for inducing inflammatory and/or auto-immune

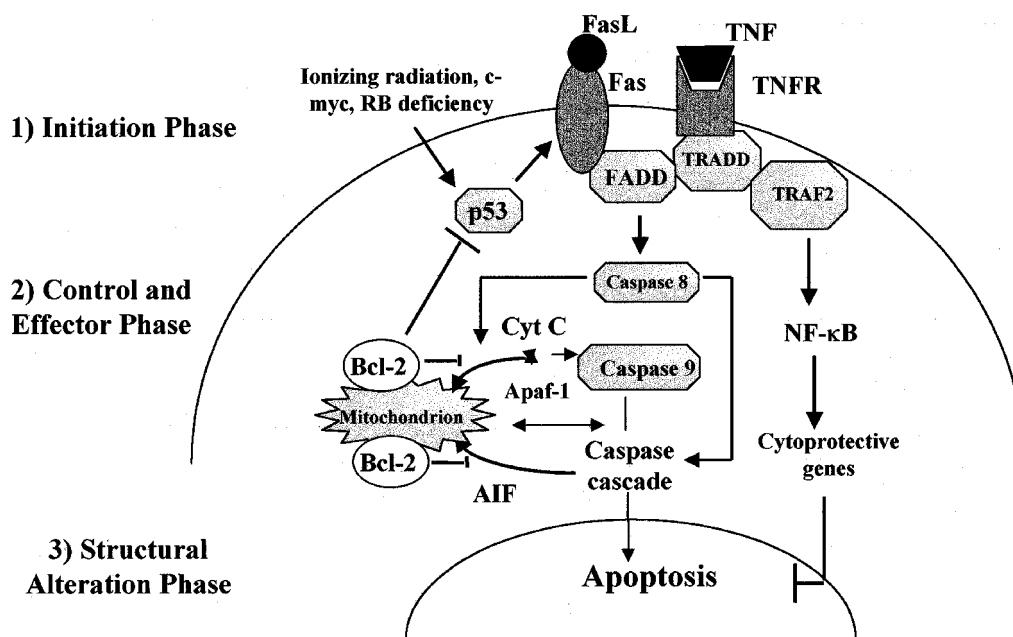
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responses (Casciola-Rosen *et al.*, 1994; Rosen *et al.*, 1995; Tan, 1994).

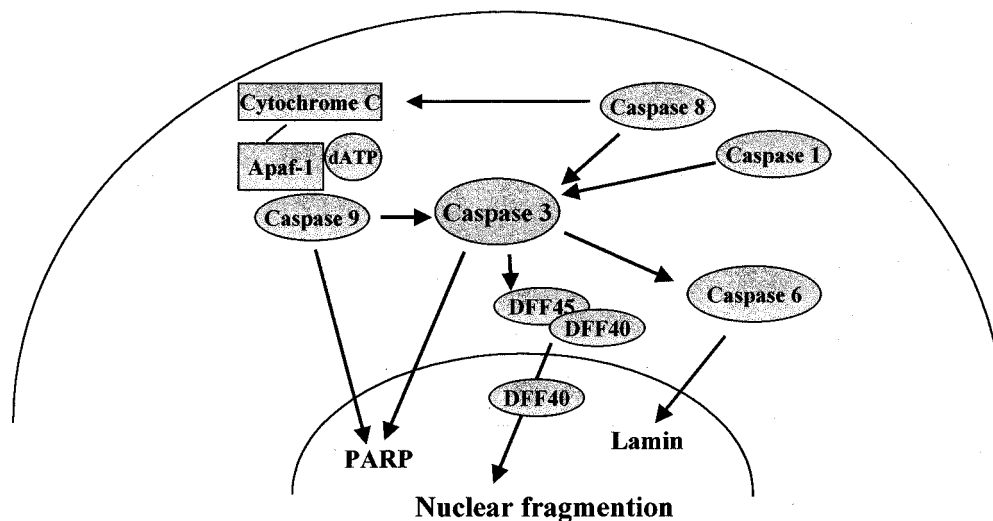
Apoptosis is controlled by an ordered, stereotyped cascade of cellular events. Some death-commitment pathways are ubiquitous (for example, those involved in radiation-induced DNA damage) while others are present only in specific cells, therefore ensuring their effective removal without the elimination of other cell types adjacent to them. It is the relative abundance of pro-apoptotic and anti-apoptotic molecules within the cell at a given time that will determine the cell's fate.

The programmed cell death cascade can be divided into at least four main functionally distinct stages. The apoptosis signalling pathways are described in more detailed reviews (Golstein, 1997; Green & Reed, 1998; Kroemer *et al.*, 1997), and will be summarized below (Figure 1):

- (1) *The initiation or signalling phase* during which cells receive the death-inducing signal. This may be accomplished by attachment of death-promoting molecules (e.g., Tumour Necrosis Factor (TNF)- $\alpha$ , Fas ligand) to death receptors (TNFR1, Fas) on the cell surface with subsequent recruitment of death domain proteins (FADD, TRADD, RIP) required for caspase-8 activation (which initiates the lethal proteolytic cascade) (Ashkenazi & Dixit, 1998).
- (2) *The control and effector phase* during which activation of caspases occurs with loss of mitochondrial membrane potential (Green & Kroemer, 1998). Caspases, the mammalian CED-3 homologues, are a family of cysteine proteases with aspartate specificity that have been implicated in the transduction and execution of the apoptotic programme (Thornberry & Lazebnik, 1998). Caspases are present as inactive pro-enzymes, most of which are activated by proteolytic cleavage. They are
- responsible for the deliberate disassembly of a cell into apoptotic bodies. Caspase-8, caspase-9 and caspase-3 are situated at pivotal junctions in apoptotic pathways (Figures 1 and 2).
- The execution phase is controlled by the Bcl-2 family members (Kroemer, 1997) acting upstream from the caspases, through inhibition of cytochrome c and/or apoptosis inducing factor (AIF) release from the mitochondrion or through binding and sequestration of Apaf-1 away from caspase-3. The Bcl-2 family of proteins contains both inhibitors (Bcl-2, Bcl-x<sub>L</sub>) and inducers (Bcl-x<sub>s</sub>, Bax, Bid, Bad, Bak) of apoptosis acting as homodimers and heterodimers. The balance between anti-apoptotic and pro-apoptotic Bcl-2 family members is critical to determining if a cell undergoes apoptosis. After an appropriate signal, Bax or Bak, for example, undergo a conformational change and move to the mitochondrial membrane where they cause release of cytochrome c into the cytosol (Goping *et al.*, 1998; Griffiths *et al.*, 1999).
- (3) *The structural alterations and DNA degradation phase* (Figure 2), where caspase activation leads to the cleaving of lamin, the intermediate filament of the nuclear envelope, of poly(ADP)ribose polymerase (PARP) (Nicholson *et al.*, 1995) and of the inhibitor of caspase-activated deoxyribonuclease (ICAD/DFF45) (Enari *et al.*, 1998; Liu *et al.*, 1997; Sakahira *et al.*, 1998). Fragmentation and degradation of genomic DNA results in an irreversible loss of viability.
- (4) *The phase of recognition of apoptotic cells and removal of apoptotic bodies*. After the completion of the apoptotic processes, dead cells are removed from the tissue as a result of specific recognition and phagocytosis by adjacent professional and/or non-professional cells through a variety of mechanisms that implicate phosphatidylserine



**Figure 1** Schematic representation of main apoptotic pathways. Activation of caspase-8 (and subsequent activation of the caspase cascade) occurs following ligation of death-signal-transmitting receptors. Caspase-9 activates cell disassembly in response to agents or insults that trigger release of cytochrome c from the mitochondria, and is activated when complexed with dATP, Apaf-1, and extramitochondrial cytochrome c to form the multi-protein 'apoptosome' ensemble. Caspase-8 and caspase-9 can activate caspase-3 by proteolytic cleavage, and caspase-3 amplifies caspase-8 and caspase-9 signals into fully-fledged commitment to disassembly, therefore propagating the caspase cascade. Ligation of the death-signal-transmitting receptor TNFR may also generate signal transduction pathways leading to activation of the nuclear transcription factor NF- $\kappa$ B and to the induction of cytoprotective genes.



**Figure 2** Schematic representation of the structural alterations and DNA fragmentation phase. DFF is composed of two subunits, DFF45 (ICAD) and DFF40 (CAD). Cleavage of DFF45 by caspase-3 activates the nuclease activity of DFF40. Other substrates for caspases include PARP and lamin.

(PS), thrombospondin/CD36 binding site, the vitronectin receptor or CD14, for example (Devitt *et al.*, 1998; Fadok *et al.*, 1992; 1998; Savill *et al.*, 1993). Loss of membrane phospholipid asymmetry and consequent exposure of PS in the exoplasmic leaflet of the plasma membrane is one of the early hallmarks of cells undergoing apoptosis, and is a critical event in their recognition by some macrophage populations (Fadok *et al.*, 1992, 1998). In addition cells undergoing apoptosis generate reactive oxygen species that may induce membrane peroxidation (Ashkenazi & Dixit, 1998). Indeed, apoptotic cells present oxidatively modified moieties on their surface that are structurally analogous to the surface of the oxidized LDL (oxLDL) particle (Chang *et al.*, 1999). These oxidation-specific epitopes, including oxidized phospholipids, serve as ligands for recognition and phagocytosis by macrophages through their scavenger receptors, including SR-A, CD36, CD68, SR-B1 (CLA-I) and LOX-1 (Bird *et al.*, 1999; Chang *et al.*, 1999). Complement activation seems to be required for efficient uptake of apoptotic cells within the systemic circulation (Mevorach *et al.*, 1998).

The transverse redistribution of plasma membrane PS is followed by the shedding of membrane particles which is a common feature of apoptotic cell death (Aupeix *et al.*, 1997). Annexin V which has a strong affinity for PS and exerts an external membrane constraint is able to reduce the degree of proteolytic activation of caspase-3 and totally blocks the release of shed membrane microparticles (Gidon-Jeangirard *et al.*, 1999).

## Apoptosis of endothelial cells

Vascular endothelial cells form the inner lining of all blood vessels. During both normal development and pathology, the formation of new vessels and the regression of preexisting ones are dependent on the balance between endothelial cell proliferation and endothelial cell apoptosis. In mature vessels, endothelial cell turnover is also under the control of these tightly regulated phenomena. Since the vascular

endothelium is involved in various physiological processes, endothelial cell apoptosis (and dysfunction) may constitute an initial step in a variety of pathological situations such as atherosclerosis and hypertension (see below). As for other cell types, it has been hypothesized that interactions of endothelial cells with their microenvironment may be critical for their survival.

### Survival pathways

Main survival pathways in endothelial cells are summarized in Table 1 and represented in Figure 3.

**Cell-cell and cell-matrix interactions** Cell-to-cell and cell-to-matrix contacts are necessary for the maintenance of survival of anchorage-dependent cells such as endothelial cells (Brooks *et al.*, 1994; Levkau *et al.*, 1998; Meredith *et al.*, 1993; Re *et al.*, 1994; Scatena *et al.*, 1998). Loss of cell-to-cell contact leads to the activation of a default death programme in a variety of cell types.

The extracellular matrix may generate survival signals aiming at the suppression of a p53-regulated cell death pathway. For example, survival signals from fibronectin are transduced by the focal adhesion kinase (FAK) whose phosphorylation leads to p53 inactivation and maintenance of cell survival (Ilic *et al.*, 1998). Interaction with extracellular matrix proteins *via* the integrin  $\alpha_v\beta_3$  inhibits p53 activity, decreases the expression of p21 and Bax (Stromblad *et al.*, 1996), and activates NF- $\kappa$ B (Scatena *et al.*, 1998), therefore promoting cell survival. This is particularly important during migration of endothelial cells in angiogenesis as well as in the maintenance of tumour vasculature (Rüegg *et al.*, 1998).

The extracellular matrix may also generate survival signals aiming at the upregulation of an anti-apoptotic pathway mainly by promoting the anti-apoptotic activity of the Bcl-2 family of proteins. Vascular endothelial cadherin, VE-cadherin, mediates adhesion between endothelial cells and affect endothelial cell survival. Deficiency or truncation of VE-cadherin induces endothelial cell apoptosis and abolishes transmission of the endothelial survival signal by VEGF-A to Akt kinase and Bcl2

**Table 1** Endothelial cell survival factors

<i>Factors promoting endothelial cell survival</i>	<i>Main transduction pathways</i>
<ul style="list-style-type: none"> <li>• <math>\alpha v\beta 3</math>-mediated cell matrix interaction</li> <li>• Fibronectin</li> <li>• VE-cadherin</li> <li>• Shear stress</li> </ul>	<p>p53, p21, bcl-2* (Stromblad <i>et al.</i>, 1996), NF-<math>\kappa</math>B activation (Levkau <i>et al.</i>, 1999; Scatena <i>et al.</i>, 1998)</p> <p>FAK activation (Ilic <i>et al.</i>, 1999)</p> <p><math>\beta</math>-catenin, PI3-K, Akt (Carmeliet <i>et al.</i>, 1999)</p> <p>Akt, NO, caspase inhibition (Dimmeler <i>et al.</i>, 1996; 1997b; Hermann <i>et al.</i>, 1997; Meeson <i>et al.</i>, 1996)</p> <p>Flk-1/KDR activation (Gerber <i>et al.</i>, 1998), VE-cadherin pathway (Carmeliet <i>et al.</i>, 1999), Protein kinase C, PI3 K (Karsan, 1998), bcl-2* (Alon <i>et al.</i>, 1995)</p> <p>PI3-K, Akt (Fujikawa <i>et al.</i>, 1999)</p> <p>+/- bcl-2* (Karsan <i>et al.</i>, 1997)</p> <p>NO (Arnal <i>et al.</i>, 1996), caspase inhibition (Alvarez <i>et al.</i>, 1997; Spyridopoulos <i>et al.</i>, 1997)</p> <p>bcl-2*? (Hancock <i>et al.</i>, 1998)</p> <p>A1 (Karsan <i>et al.</i>, 1996)</p> <p>bcl-2* (Noble <i>et al.</i>, 1999)</p>
<ul style="list-style-type: none"> <li>• VEGF</li> </ul>	
<ul style="list-style-type: none"> <li>• Angiopoietin-1</li> <li>• FGF-2</li> <li>• Estradiol</li> </ul>	
<ul style="list-style-type: none"> <li>• Haeme oxygenase-1</li> <li>• TNF-<math>\alpha</math></li> <li>• Direct contact with monocytes</li> </ul>	

\*bcl-2 and/or bcl-2 family members.

via reduced complex formation with VEGF receptor-2,  $\beta$ -catenin, and phosphoinositide 3 (PI3)-kinase (Carmeliet *et al.*, 1999). Growth factor deprivation of endothelial cells induces apoptosis, and this correlates with cleavage and disassembly of intracellular and extracellular components of adherens junctions. Caspase-3 appears to initiate proteolytic processing, leading to the cleavage of FAK (Levkau *et al.*, 1998),  $\beta$ -catenin and plakoglobin and to the shedding of VE-cadherin (Herren *et al.*, 1998), actively interrupting extracellular signals required for endothelial cell survival.

**Growth factors** *In vitro*, VEGF inhibits endothelial cell apoptosis in response to a variety of stimuli such as hyperoxia (Alon *et al.*, 1995), growth factor withdrawal, disruption of extracellular matrix (Watanabe & Dvorak, 1997), ionizing radiation (Kato *et al.*, 1995), peroxynitrite and stimulation with TNF- $\alpha$  (Karsan, 1998). This is accomplished in part through interaction with adherens junctions (Carmeliet *et al.*, 1999), stimulation of protein kinase C or PI3K-Akt signalling pathway (Karsan, 1998), upregulation of the anti-apoptotic proteins Bcl-2 and A1 (Gerber *et al.*, 1998), or those of the inhibitors of apoptosis family of anti-apoptotic proteins, i.e. survivin and XIAP (Tran *et al.*, 1999).

Angiopoietin-1 ligation to its receptor Tie-2 has recently been shown to provide survival signals for the endothelial cell (Fujikawa *et al.*, 1999; Hayes *et al.*, 1999; Kwak *et al.*, 1999). Growth-deprived endothelial cells are rescued from apoptosis by addition of angiopoietin-1. Angiopoietin-1 induces Tie-2 autophosphorylation leading to PI3K-dependent activation of Akt. The anti-apoptotic effect of angiopoietin-1, as that of VEGF, is mediated through interaction with the extracellular matrix since the anti-apoptotic effect of these growth factors is lost in suspended cells (Fujikawa *et al.*, 1999). Angiopoietin-1 expression is associated with tumour neovascularization and tumour growth *in vivo* (Holash *et al.*, 1999).

Basic fibroblast growth factor (FGF-2) is another intravascular survival factor for endothelial cells *in vivo*. Intravenous administration of FGF-2 in a murine model of endotoxic shock blocks LPS-induced ceramide generation and endothelial cell apoptosis. Consequently, animal mortality is reduced despite persistent elevation in serum TNF- $\alpha$  (Haimovitz-Friedman *et al.*, 1997). *In vitro* studies have shown that FGF-2 specifically induces Bcl-2, but not other members of the

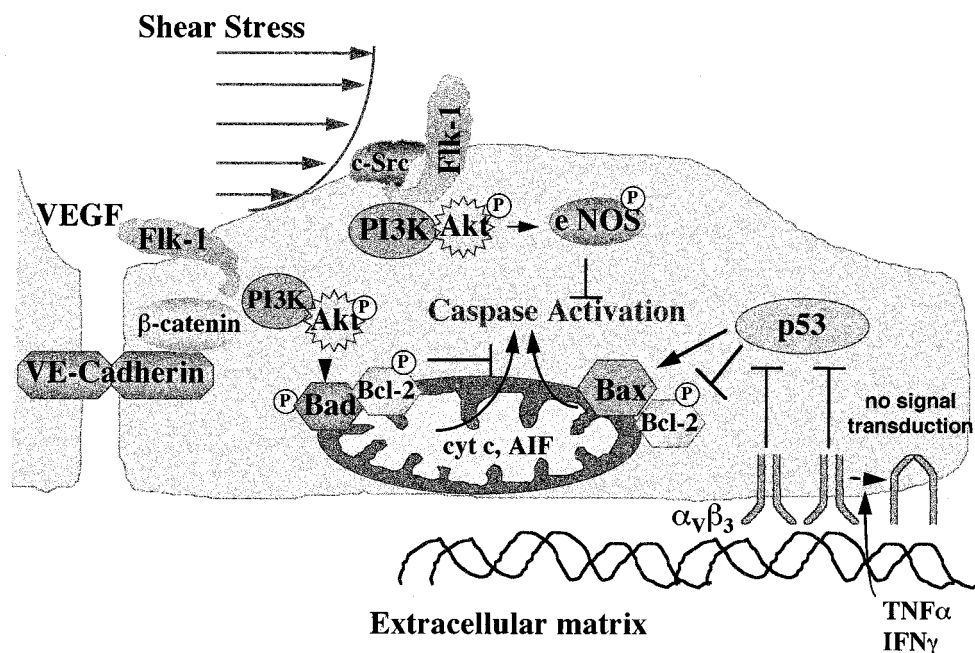
Bcl-2 family, and inhibits serum-deprivation-induced endothelial cell death (Karsan *et al.*, 1997). However, other Bcl-2-independent mechanisms, such as tyrosine phosphorylation, may also account for the inhibition of endothelial apoptosis by FGF-2 (Karsan *et al.*, 1997).

**Shear stress** Vascular endothelial cells are continuously exposed to a range of haemodynamic forces which have a great impact on their cellular structure and function. Variations in blood flow play an important role in vessel growth or regression, and in the focal development of atherosclerosis. A link between mechanical stimulation and cell survival or death has been therefore suggested by several groups. Indeed, human umbilical vein endothelial cells (HUVEC) cultured under static conditions undergo a basal level of apoptosis (Kaiser *et al.*, 1997). The empty space left between cells induces proliferation of adjacent cells so that an equilibrium state is reached with low levels of apoptosis and proliferation. Exposure to flow in a perfusion chamber or in an *ex vivo* organ culture directly inhibits the apoptotic process and indirectly suppresses proliferation, leading to a 'truly' quiescent monolayer (Kaiser *et al.*, 1997).

This anti-apoptotic pathway involves the shear-induced phosphorylation of Akt/PKB (Dimmeler *et al.*, 1998), and the subsequent phosphorylation of the endothelial NO synthase (Dimmeler *et al.*, 1999a). NO, released in response to shear stress, inhibits caspase-3 activation and prevents endothelial cell apoptosis (Dimmeler *et al.*, 1997b).

The suppression of both endothelial cell apoptosis and caspase-3 activation by physiological levels of shear stress in other models (growth factor withdrawal or cytokine-induced apoptosis) may be prevented by pharmacological inhibition of glutathione biosynthesis or nitric oxide synthase (Dimmeler *et al.*, 1996; Hermann *et al.*, 1997). Shear stress-dependent upregulation of Cu/Zn SOD and NO synthase prevents activation of the caspase cascade in response to apoptotic stimuli, including oxygen free radicals, oxLDL or TNF $\alpha$  (Dimmeler & Zeiher, 1999). This suggests that the reduction in oxidative flux by shear stress may be essential to prevent the activation of caspases and to inhibit endothelial cell apoptosis.

The modulation of endothelial cell apoptosis by shear stress plays a major role in normal and pathological vascular remodelling *in vivo* (see below).



**Figure 3** Schematic drawing that shows the importance of cell-to-cell and cell-to-matrix-interactions in endothelial cell survival. The survival effect of growth factors like VEGF is dependent on cell-to-cell contact (involving VE-cadherin pathway). VE-cadherins can be upregulated by shear stress. Akt phosphorylation is central to many anti-apoptotic signalling pathways and modulates the balance between anti-apoptotic and pro-apoptotic bcl-2 family members. Pro-inflammatory stimuli, including TNF- $\alpha$  and IFN- $\gamma$ , may interfere with matrix-dependent cell survival pathways.

### Apoptotic pathways

A number of extra- or intracellular agents can induce apoptosis in endothelial cells as a result of loss of survival factors (see above), modulation of survival pathways or induction of pro-apoptotic pathways.

**Modulation of survival pathways** Some potentially important pro-apoptotic factors act essentially by inhibiting survival pathways. For example, exposure of human endothelial cells to TNF- $\alpha$  and IFN- $\gamma$  results in suppression of endothelial cell  $\alpha_v\beta_3$  activity leading to a decreased  $\alpha_v\beta_3$ -dependent endothelial cell adhesion and survival (Rüegg *et al.*, 1998). *In vivo*, TNF- $\alpha$  and IFN- $\gamma$  interact with  $\alpha_v\beta_3$  to cause selective disruption of the tumour vasculature in patients with melanoma (Rüegg *et al.*, 1998).

Stimulation of human endothelial cells with TNF- $\alpha$  leads to a specific degradation of Bcl-2, a process that is inhibited by specific proteasome inhibitors (Dimmeler *et al.*, 1999b).

Finally, angiostatin-induced endothelial cell apoptosis (Claesson-Welsh *et al.*, 1998; Lucas *et al.*, 1998) paradoxically involves FAK activation (Claesson-Welsh *et al.*, 1998). FAK activation by angiostatin is not associated with Src coactivation and does not involve integrin signalling. In fact, angiostatin probably causes a flawed, integrin-independent activation of FAK that perturbs the ordered turnover of focal adhesion contacts induced by VEGF and FGF-2. Angiostatin-induced endothelial apoptosis may be an important mechanism by which angiostatin modulates the angiogenic process.

### Induction of pro-apoptotic pathways

**Inflammatory mediators** Endothelial dysfunction is central to many inflammatory diseases affecting the vessel wall (atherosclerosis, for example) and several other organs (septic shock). Also, inflammatory cytokines such as TNF- $\alpha$  are increased in

the circulating blood of patients with terminal heart failure and may participate to the vascular dysfunction observed in this disease. Incubation of HUVEC with TNF- $\alpha$  markedly increases endothelial cell apoptosis *via* activation of caspase-3, a process that can be completely abrogated by inhibitors of caspases or by shear stress (Dimmeler *et al.*, 1996).

Peripheral blood mononuclear monocytes (PBMCs) pre-activated by bacterial lipopolysaccharide (LPS) or by ionizing radiation induce apoptotic death in cultured HUVECs (Lindner *et al.*, 1997). This process is dependent at least in part on TNF- $\alpha$  since addition of an anti-TNF- $\alpha$  monoclonal antibody blocks the cell-to-cell contact-mediated apoptosis. Interestingly, the anti-inflammatory cytokine IL-10 has anti-apoptotic effects in this model, suggesting that the balance between endothelial cell survival and death may depend on the balance between pro- and anti-inflammatory cytokines. Incubation of HUVEC with LPS in culture induces apoptosis and secondary necrosis in a time-dependent manner. In this setting, apoptosis is independent of TNF- $\alpha$  release but is associated with the induction of Bax and is prevented by incubation with antioxidants (that enhance Bcl-2 and decrease Bax) (Haendeler *et al.*, 1996). It is noteworthy that TNF- $\alpha$ , like other inflammatory cytokines, is capable of inducing human A1, a human Bcl-2 homologue (Karsan *et al.*, 1996) and may also activate the NF- $\kappa$ B pathway (Van Antwerp *et al.*, 1996). TNF- $\alpha$  may therefore initiate both pro-apoptotic and anti-apoptotic pathways.

Systemic administration of LPS induces the endotoxic shock syndrome with associated systemic inflammation, multiple organ damage, circulatory collapse and death. This process is mediated by the release of TNF- $\alpha$  and other cytokines. Since TNF- $\alpha$  and LPS can cause apoptotic death of endothelial cells, it has been hypothesized that the primary tissue target in septic shock may be the endothelium. Indeed, it was demonstrated that injection of LPS and TNF- $\alpha$  in mice induces massive apoptosis in the endothelium of several

organs before any other form of tissue damage (Haimovitz-Friedman *et al.*, 1997). Unlike studies performed *in vitro*, LPS-induced endothelial apoptosis *in vivo* is dependent on TNF- $\alpha$  since it is blocked by the TNF-binding protein. This latter molecule also prevents LPS-induced ceramide generation, suggesting that systemic TNF is required for both responses (Haimovitz-Friedman *et al.*, 1997). The indispensable role of ceramide generation in disseminated endothelial apoptosis is demonstrated by the observation that acid sphingomyelinase knockout mice are protected from endothelial cell apoptosis and from death despite the normal increase in serum TNF- $\alpha$  in response to LPS (Haimovitz-Friedman *et al.*, 1997). It would be interesting to examine whether the beneficial effects of IL-10 in reducing mortality from septic shock in animals (Howard *et al.*, 1993) are related to its potential anti-apoptotic properties.

In the context of endothelial inflammation, it is particularly important to point out that the antiapoptotic proteins Bcl-2 and Bcl-X<sub>L</sub> are able to downregulate endothelial cell activation through specific inhibition of NF- $\kappa$ B at a level upstream of I $\kappa$ B $\alpha$  degradation (Badrichani *et al.*, 1999). Bcl-2 and Bcl-X<sub>L</sub> may therefore be cytoprotective in endothelial cells by counteracting both proapoptotic and proinflammatory insults.

**Oxidized lipoproteins** The evidence available from several groups suggests that oxLDL play a central role in atherogenesis beginning in the earliest phases of this threatening process. *In vitro* studies had already shown that oxLDL exhibit cytotoxic effects on cultured endothelial cells, but the type of cell death elicited remained unknown (Hessler *et al.*, 1979). However, several authors recently reported increased apoptotic cell death of bovine aortic endothelial cells exposed to cholesterol oxides in culture (Lizard *et al.*, 1996) and increased apoptosis (and possibly secondary necrosis) of HUVEC after exposure to oxLDL (Dimmeler *et al.*, 1997a; Escargueil-Blanc *et al.*, 1997). OxLDL-induced apoptosis is subsequent to a sustained and delayed peak in cytosolic calcium and is prevented by chelating extracellular calcium or by inhibiting calcium influx (Escargueil-Blanc *et al.*, 1997). Furthermore, ceramide generation by acid sphingomyelinase and activation of caspase-3 are indispensable for oxLDL-induced apoptosis (Harada-Shiba *et al.*, 1998). OxLDL are known to increase the generation of reactive oxygen species which are implicated in the apoptotic process in various cell types. Addition of antioxidants (vitamins C and E) completely prevents the activation of caspase-3 by oxLDL and inhibits the apoptotic process (Dimmeler *et al.*, 1997a). Although vascular endothelial cells are normally resistant to Fas-mediated cell apoptosis (Richardson *et al.*, 1994), apoptosis induced by OxLDL in cultured endothelial cells and endothelium of arterial explants appears to be inhibited by Fas ligand neutralizing antibodies, suggesting that OxLDL may promote Fas-mediated endothelial cell apoptosis (Sata & Walsh, 1998).

## Apoptosis of vascular smooth muscle cells

After decades of intensive research on the mechanisms of vascular smooth muscle cell (VSMC) proliferation, recent studies have been undertaken to understand the mechanisms and roles of smooth muscle cell survival/apoptosis in normal vessel development and pathology. VSMC growth is now viewed as the result of the opposing effects of cell proliferation and apoptosis.

## Survival pathways

**Cell-cell and cell-matrix interactions** As for endothelial cells, cell-matrix interactions are necessary for vascular smooth muscle cell homeostasis through the generation of signals involved in cell survival, migration and/or proliferation. Fibronectin and  $\alpha_v\beta_3$  play important roles in the maintenance of these transduction pathways (Coleman *et al.*, 1999; Dufourcq *et al.*, 1998; Mason *et al.*, 1999; Zheng & Clemmons, 1998). The matrix glycoprotein tenascin-C has also been shown to modulate smooth muscle cell survival and proliferation. Tenascin-C interactions with  $\alpha_v\beta_3$  integrins upon upregulation by denatured type I collagen modify VSMC shape, and epidermal growth factor (EGF)-dependent growth by inducing a clustering of the EGF-receptors (Jones *et al.*, 1997a). These extracellular matrix-dependent survival pathways have been shown to be involved in the progression of pulmonary vascular diseases (Jones *et al.*, 1997b) and are likely to play an important role in VSMC survival within the atherosclerotic plaque.

**Growth factors** Many known growth factors for VSMCs (IGF-1, PDGF-BB, FGF-2 and TGF- $\beta$ 1) have been shown to act as survival factors rather than true mitogens. These growth factors partially prevent apoptosis of human or rat VSMCs cultured under low serum conditions (Bai *et al.*, 1999; Bennett *et al.*, 1995a; Fox & Shanley, 1996; Pollman *et al.*, 1999a) in part by favouring the anti-apoptotic potential of the Bcl-2 family of proteins. For example, IGF-I stimulates increased expression of the inactive, phosphorylated form of Bad by a PI3-kinase-dependent pathway (Bai *et al.*, 1999). These growth factors and their corresponding receptors are expressed in many vascular diseases and may prevent VSMC death within the arterial wall.

## Apoptotic pathways

Many pro-apoptotic factors for VSMCs have been identified, some with potential implications in many vascular diseases such as atherosclerosis, restenosis or hypertension.

**Mechanical factors** Balloon angioplasty induces rapid death of VSMCs within the normal arterial wall (Pollman *et al.*, 1999b). VSMC apoptosis is associated with the induction of a redox-sensitive signalling pathway leading to the activation of the stress-activated protein kinase (SAPK). Administration of anti-oxidants inhibits SAPK activation and VSMC apoptosis. This signalling pathway could not be activated in neointimal smooth muscle cells due to the upregulation of the antiapoptotic mediator bcl-x<sub>L</sub> in these cells (Pollman *et al.*, 1999b).

**Oxidized LDL** Oxidized LDL (Jorjine *et al.*, 1997) and oxysterols (Nishio & Watanabe, 1996) have been shown to promote VSMC apoptosis in culture, in part by down-regulation of Bcl-2 and activation of caspase-3 (Nishio & Watanabe, 1996).

**Vasoactive substances** Local production of high levels of NO within the arterial wall inhibits cell growth and neointimal formation following balloon injury (von der Leyen *et al.*, 1995), while forced local generation of Ang II in the vessel wall (after overexpression of the angiotensin-converting enzyme, ACE) promotes VSMC growth and wall thickening (Morishita *et al.*, 1994). These effects on vascular remodelling are likely the result of a modulation of the

apoptotic process in VSMCs. NO-induced apoptosis in VSMCs is prevented by the inhibition of the cGMP-dependent protein kinase I $\alpha$ , as well as by the addition of Ang II (acting *via* the angiotensin receptor type 1), emphasizing the importance of the balance between NO and Ang II in the modulation of VSMC growth (Pollman *et al.*, 1996). It is noteworthy that the pro-apoptotic effects of NO occur at supraphysiological (pathological or inflammatory) levels, while its anti-apoptotic effects, through the prevention of caspase activation, are essentially observed at physiological (endothelial) concentrations (Dimmeler *et al.*, 1997b; Kim *et al.*, 1999). Similarly, Ang II may have dual effects on VSMC apoptosis, preventing cell death *via* AT1 receptor stimulation, but promoting apoptosis *via* AT2 receptor stimulation (Yamada *et al.*, 1998). Yet, the AT2 receptor is expressed abundantly in foetus, but scantily in adult tissues. Therefore it might play a major role in vascular remodelling during development but its role in adult remains to be evaluated.

**Inflammatory mediators** Production of high levels of NO via the inducible nitric oxide synthase (iNOS) accounts for the pro-apoptotic effects of pro-inflammatory cytokines (TNF- $\alpha$ , IFN- $\gamma$  and IL-1) in rat but not human VSMCs (Geng *et al.*, 1996). Stimulation of VSMCs in culture by pro-inflammatory cytokines also induces the expression of Fas (and increases their sensitivity to apoptosis) (Geng *et al.*, 1997), suggesting that the production of such cytokines by immune cells in atherosclerosis may promote VSMC death through this pathway. Inflammatory cells, particularly macrophages, have the potential of inducing VSMC apoptosis in culture. This process is dependent on cell-to-cell contact and on TNF $\alpha$  and FasL interactions with their corresponding receptors TNFR-I and II, and Fas (Boyle *et al.*, 1998).

**Oncogenes and tumour suppressor genes** Deregulated expression of the *c-myc* oncogene and stable infection with the adenovirus E1A induce apoptosis in rat VSMCs under low serum conditions (Bennett *et al.*, 1994, 1995a). Moreover, overexpression of p53 induces apoptosis in rat VSMCs infected with *c-myc* and E1A, but not in normal cells (Bennett *et al.*, 1995a).

These observations are important for several reasons: the *c-myc* oncogene is overexpressed in VSMCs from human atherosclerotic plaques and these cells display a lower growth rate in culture in comparison with VSMCs obtained from normal arteries, especially under low serum conditions (Parkes *et al.*, 1991). As expected, this intrinsic defect in growth has recently been shown to be, at least in part, the result of increased spontaneous cell death by apoptosis (Bennett *et al.*, 1995b). The characteristics of plaque VSMCs may also explain in part their hypersensitivity to p53-mediated apoptosis (Bennett *et al.*, 1997). p53 activation in cells derived from atherosclerotic plaque transiently increases surface Fas expression by transport from the Golgi complex, and induces Fas-FADD binding, sensitizing cells to Fas-induced apoptosis (Bennett *et al.*, 1998b). However, basal p53 activity is similar in plaque and normal VSMCs and suppression of basal p53 activity blocks growth arrest in both cell types, but does not prevent apoptosis suggesting that the mechanisms of p53-mediated apoptosis of plaque VSMCs may be distinct from those inducing growth arrest (Bennett *et al.*, 1997). The slower rates of proliferation and earlier senescence of plaque VSMCs may be explained by the predominance of the hypophosphorylated form of the retinoblastoma gene product (RB) and the low E2F

transcriptional activity in these cells (Bennett *et al.*, 1998a). Both inactivation of RB and inhibition of p53 activity are required for plaque VSMCs to proliferate without apoptosis (Bennett *et al.*, 1998a).

VSMC apoptosis may be induced *in vitro* by many factors including inhibitors of protein kinase C, calcium channel blockers, angiotensin-converting enzyme inhibitors, stimulators of cyclic AMP-dependent protein kinase (Leszczynski *et al.*, 1994), and tissue inhibitor of metalloproteinase type 3 (Baker *et al.*, 1998). However, the clinical relevance of the pro-apoptotic effects of these factors remains to be established.

## Role of apoptosis in normal vessel development

Physiological remodelling of blood vessels before and after birth has been shown to be the result of a balance between apoptosis and cell proliferation. Developmentally programmed capillary regression is initiated by macrophage-mediated endothelial cell apoptosis (Lang & Bishop, 1993). The dying cells are projected into the capillary lumen causing temporary or permanent block to blood flow. Following cessation of flow, secondary synchronous apoptosis of vascular endothelial cells occurs, leading to capillary regression (Meeson *et al.*, 1996). It can be hypothesized that during development, the selection of capillary beds is under the control of blood flow-dependent endothelial cell survival/apoptosis.

Apoptosis of smooth muscle cells occurs during the development of the human ductus arteriosus (Slomp *et al.*, 1997). The role of apoptosis has also been investigated in vessel remodelling that occurs as arteries adapt to changes in cardiovascular function after birth. The abdominal aorta of neonatal lambs undergoes excessive remodelling in association with a profound decrease in blood flow after birth. However, DNA accumulation in this vessel is far below that predicted from proliferation rates if all cells were assumed to survive. Cho *et al.* (1995) have demonstrated that apoptosis significantly contributes to postpartum arterial remodelling and that changes in cell death rates alone may be sufficient to induce profound changes in the vessel wall mass (Cho *et al.*, 1995).

## Role of apoptosis in vascular pathology

Apoptosis appears to play a significant role in many vascular or vascular-related diseases. In this review, a particular emphasis is put on the potential role of apoptosis in atherosclerosis and restenosis. Reviews on the occurrence and role of apoptosis in hypertensive diseases have been recently published (Diez *et al.*, 1998; Hamet *et al.*, 1996).

### *Apoptosis in atherosclerosis*

Observations that cell death occurs in atherosclerosis have already been made by Virchow (1858), and experimental studies in cholesterol-fed swines undertaken by Thomas *et al.* (1976) more than 20 years ago showed that cell death is a major event occurring during atherosclerotic plaque development and may be associated with cell proliferation. More recently, Parkes *et al.* observed that plaque-derived smooth muscle cells (p-VSMC) are characterized by *c-myc* overexpression and have a growth disadvantage in culture in comparison with VSMCs from normal arteries, especially under low serum conditions (Parkes *et al.*, 1991). At that time,

cancer researchers have shown that overexpression of *c-myc* induced cell death by apoptosis in the absence of serum and that cells can be rescued by overexpression of *bcl-2* (Evan *et al.*, 1992). On the basis of these results, it has been suggested that cell death by apoptosis may occur in human atherosclerosis. Bennett *et al.* have subsequently demonstrated that deregulated expression of *c-myc* in VSMCs induces apoptosis (Bennett *et al.*, 1994) and that human p-SMCs have a markedly elevated rate of apoptosis *in vitro*, a process that can be reversed by expression of *bcl-2* or by other survival factors such as IGF-1 and PDGF (Bennett *et al.*, 1995b). This was the first indication that programmed cell death (apoptosis) may occur in human atherosclerosis.

### In situ distribution of apoptosis

Several studies have shown evidence for *in situ* apoptotic cell death in animal and human atherosclerotic plaques (Björkerud & Björkerud, 1996; Cai *et al.*, 1997; Geng & Libby, 1995; Han *et al.*, 1995; Hegyi *et al.*, 1996; Isner *et al.*, 1995; Kockx *et al.*, 1996; Mallat *et al.*, 1997). Apoptosis, which is almost absent in normal arteries, becomes barely detectable in fatty streaks and is more abundant in advanced plaques. All cell types are involved, including SMCs, macrophages, T-lymphocytes (Björkerud & Björkerud, 1996; Cai *et al.*, 1997; Geng & Libby, 1995; Han *et al.*, 1995; Hegyi *et al.*, 1996; Isner *et al.*, 1995; Kockx *et al.*, 1996; Mallat *et al.*, 1997) and luminal endothelial cells (Tricot *et al.*, 2000). Removal of apoptotic cells in human plaques may be inefficient, as a significant percentage of secondary necrosis is observed. Foam cell apoptosis (and secondary necrosis) is thought to contribute to the formation of the acellular lipid core (Cai *et al.*, 1997), whereas VSMCs die within their cages of thickened basal lamina in the hypocellular fibrous cap and are split into a myriad of membrane vesicles (Kockx *et al.*, 1998). The distribution of apoptosis is heterogeneous within the plaque, being more frequent in regions with a high density in macrophages suggesting that these cells may be participating in the induction of apoptosis. In addition, we have recently observed that luminal endothelial apoptosis is directly related to the blood flow direction, being more prevalent in the post-stenotic area where low flow and low shear stress prevail (Tricot *et al.*, 2000). This *in vivo* finding in human atherosclerotic plaques confirms the *in vitro* observation of a strong modulation of endothelial cell survival by shear stress as indicated above.

### Pro- and anti-apoptotic factors in atherosclerotic plaques

Many factors with *in vitro* pro- and anti-apoptotic activities in macrophages, smooth muscle cells and endothelial cells have been shown to be expressed in human atherosclerosis (Table 2). Pro-inflammatory cytokines with pro-apoptotic potential including IL-1, TNF- $\alpha$ , and IFN- $\gamma$  are present in the human plaque and may be involved in the local induction of the apoptotic process, although no studies have been performed showing direct correlation between their expression and the occurrence of apoptosis. However, one of their mediators, inflammatory NO, produced as the result of upregulation of iNOS, may exert potent cell cytotoxicity. We have recently found a positive association between iNOS expression and TUNEL labelling for apoptotic cells in advanced human atherosclerosis (Mallat *et al.*, 1999a).

On the other hand, high levels of expression of the anti-inflammatory cytokine IL-10 are associated with significantly reduced levels of iNOS expression and apoptotic cell death (Mallat *et al.*, 1999a), suggesting that apoptosis may result from an excessive inflammatory reaction and may be modulated by interfering with the inflammatory response.

Cytokine-induced apoptosis may be mediated in part by the Fas receptor. *In vitro* studies have shown that pro-inflammatory cytokines induce upregulation of Fas in human VSMCs and this fits well with the more prominent expression of Fas in the human plaque intima than in the media (Geng *et al.*, 1997). Moreover, cytokine-primed VSMCs, but not unprimed cells, are sensitive to anti-Fas stimulation *in vitro* and undergo apoptosis. This may explain the association between Fas-positive cells and apoptotic VSMCs in regions of plaques that are rich in activated T-lymphocytes and macrophages (Geng *et al.*, 1997). Fas-mediated apoptotic cytotoxicity was also reported in human transplant coronary artery disease (Dong *et al.*, 1996). Interestingly, decreased Fas expression in a subset of plaque-SMCs has been reported, which could lead to resistance to apoptosis *via* the Fas system (Han *et al.*, 1996).

Smooth muscle cells die within the human plaque despite the presence of survival factors such as IGF-1 and PDGF (Bennett *et al.*, 1995b). Deregulated expression of *c-myc* in the absence of significant Bcl-2 expression (Bennett *et al.*, 1994), and increased sensitivity to p53 (Bennett *et al.*, 1997) might offer some explanations. Recent data have also shown increased expression of the pro-apoptotic protein Bax in human fatty streaks and advanced plaques. Bax was undetectable in normal arteries where expression of the anti-apoptotic protein Bcl-x<sub>L</sub> predominated (Kockx *et al.*, 1998).

**Table 2** Pro- and Anti-apoptotic factors in human atherosclerotic plaques

<i>Pro-apoptotic</i>	<i>Anti-apoptotic*</i>
<ul style="list-style-type: none"> <li>• Oxidized LDL, oxysterols and other oxidation products</li> <li>• Low shear stress</li> <li>• Matrix degradation by MMPs→cell detachment</li> <li>• Pro-inflammatory mediators: TNF-<math>\alpha</math>, IFN-<math>\gamma</math>, IL-1, inflammatory NO...</li> <li>• Vasoactive substances: ang II (for endothelial cells)</li> <li>• Fas-Fas L signalling</li> <li>• Pro-apoptotic Bcl-2 family members Bax, Bcl-x<sub>S</sub>...</li> <li>• Executioners of the apoptotic process: caspases, perforin, granzyme B</li> </ul>	<ul style="list-style-type: none"> <li>• Anti-oxidants (HO-1, <math>\alpha</math>-tocopherol...)</li> <li>• Normal or high shear stress→NO production, superoxide dismutase...</li> <li>• Adhesion proteins: <math>\alpha</math>v<math>\beta</math>3, cadherins...</li> <li>• Anti-inflammatory cytokines: e.g., IL-10</li> <li>• Growth factors: ang II (for VSMCs), PDGF, IGF-1, FGF2, VEGF...</li> <li>• Bcl-2?, Bcl-x<sub>L</sub></li> </ul>

\*The balance between pro-apoptotic and anti-apoptotic factors appears to be in favour of the former.



Therefore, the balance between pro- and anti-apoptotic proteins in atherosclerosis is in favour of the former, suggesting that plaque-SMCs are programmed to die and undergo apoptosis when additional pro-apoptotic stimuli are present (inflammatory cells and cytokines).

Cell survival within human atherosclerotic plaques may also depend on cell interactions with the extracellular matrix. Matrix metalloproteinases (MMPs) are overexpressed in human plaques and play an important role in matrix degradation (Galis *et al.*, 1994). This may result in cell detachment from the matrix and in induction of apoptosis. This hypothesis is supported by the finding that overexpression of the tissue inhibitor of metalloproteinases (TIMP-1) in the arterial wall of ApoE Knockout mice is associated with an increased density of smooth muscle cells within the atheromatous plaque (Rouis *et al.*, 1999). On the other hand, nonfibrillar or monomeric collagen (present in atherosclerotic plaques) allows VSMCs to undergo proliferation in response to mitogens in culture, while fibrillar collagen (present in the normal media of arteries) inhibits cell proliferation by up-regulating specific inhibitors of the cell cycle (Koyama *et al.*, 1996). Thus, the matrix that surrounds the cells is not neutral and may determine whether they remain quiescent, survive or multiply in response to growth factors (Ross, 1999).

The execution phase of apoptosis is caspase-dependent. In accordance with this concept, we and others (Geng & Libby, 1995; Mallat *et al.*, 1997) have shown that caspases, especially caspase-3, colocalized with apoptotic cells, particularly macrophages, in advanced human atherosclerotic plaques. However, caspase-3 positive, but TUNEL-negative, T-lymphocytes may also be observed. This is consistent with the observation that caspase-3 cleavage in T-cells may occur without the induction of apoptosis (Miossec *et al.*, 1997).

#### *Potential roles of apoptosis in human atherosclerosis (Table 3)*

Much interest has focused on the regulation of apoptosis, but only speculations have been raised about the potential roles, beneficial or harmful, of this process of cell death in atherosclerosis. Death of VSMCs by apoptosis may be viewed as harmful because it may weaken the fibrous cap by decreasing the synthesis of extracellular matrix and therefore may lead to plaque rupture. However, others may argue that plaque stabilization observed with anti- $\beta_3$  integrin antibodies (Topol *et al.*, 1994) may involve selective apoptosis of intimal smooth muscle cells that express  $\alpha_v\beta_3$  integrin and whose attachment to the extracellular matrix are disrupted.

Death of T-lymphocytes and macrophages by apoptosis may be viewed as beneficial if apoptosis is not accompanied by an inflammatory reaction. Indeed, removal of these cells from

the plaque could attenuate the inflammatory response, decrease the synthesis of MMPs and the consequent breakdown of the extracellular matrix, therefore favouring plaque stabilization. It may also be argued that apoptotic death of any cell type in the plaque may favour plaque regression over plaque progression. However, the rate of apoptotic cell death in the plaque (2–10%) (Bauriedel *et al.*, 1998; Kockx *et al.*, 1996; Mallat *et al.*, 1997) may, in many instances, exceed the reported rate of cell proliferation (<1%) (Brandl *et al.*, 1997; Gordon *et al.*, 1990). In the light of observations that human atherosclerotic plaques are generally more prone to progression rather than regression, it can be suggested that apoptosis is actually implicated in natural plaque progression (rather than regression), through development of the acellular lipid 'necrotic' core. Indeed, macrophage apoptosis has frequently been identified at the edges of the lipid core, suggesting that it may actively contribute to its formation. Moreover, the heterogeneous distribution of apoptosis implies that some regions of the plaque may show substantially higher levels of cell death, possibly predisposing to plaque rupture (and vessel occlusion or plaque progression). Removal of apoptotic cells from the atherosclerotic tissue may not be efficient because apoptotic cells may compete with oxidized lipids for removal by macrophages (Chang *et al.*, 1999). This would lead to the persistence of apoptotic bodies with potentially high immunogenic properties (Levine & Koh, 1999). Moreover, unremoved apoptotic cells are prone to undergo secondary necrosis and this may lead to accumulation of extracellular lipids and to perpetuation of the inflammatory response.

We believe that one of the major roles of apoptosis in atherosclerosis is related to its procoagulant potential. PS exposure on the outer cell surface, a hallmark of apoptosis, greatly enhances tissue factor (TF) activity and is therefore highly thrombogenic (Bach *et al.*, 1986). This procoagulant potential of apoptotic cells may be deleterious in atherosclerosis since it may be involved in determining plaque thrombogenicity following plaque rupture and may therefore favour the occurrence of acute ischaemic events and infarction. Indeed, Flynn *et al.* (1997) have shown that thrombin generation is increased at the contact of plaque-derived smooth cells undergoing apoptosis in culture. Moreover, we have recently observed significant extracellular TF expression in and around apoptotic cells in some regions of human atherosclerotic plaques suggesting that TF may be shed from apoptotic cells *via* apoptotic microparticles (Mallat *et al.*, 1999b). To examine whether apoptosis may be involved in TF activity *in vivo*, we examined advanced human atherosclerotic plaques for the presence of shed membrane apoptotic microparticles (captured by biotinylated annexin V insolubilized onto streptavidin-coated microtitration plaques) and determined the procoagulant potential of these microparticles

**Table 3** Potential roles of apoptosis in atherosclerosis

<i>Beneficial effects</i>	<i>Deleterious effects</i>
<ul style="list-style-type: none"> <li>● Decreased inflammation</li> <li>● Diminished macrophage pool</li> </ul>	<ul style="list-style-type: none"> <li>● Weakening of the fibrous cap (apoptosis of VSMCs)</li> <li>● Increased lipid core and gruel formation (inefficient removal of apoptotic cells and bodies)</li> <li>● Chemotactic/growth-stimulating properties of apoptotic debris</li> <li>● Revelation of cryptic antigens (→autoimmunity)</li> <li>● Procoagulant potential (→plaque thrombosis following plaque rupture)</li> <li>● Plaque erosion/thrombosis (luminal endothelial cell apoptosis)</li> </ul>

(Mallat *et al.*, 1999b). We found that high levels of shed membrane microparticles of monocytic and lymphocytic origins are produced in the atherosclerotic plaques and are associated with increased TF activity. Interestingly, removing the microparticles from the supernatants resulted in a 97% reduction in TF activity, indicating that almost all of the TF activity of plaque extracts is associated with the shed membrane microparticles (Mallat *et al.*, 1999b). These experiments provide direct evidence that apoptosis is involved in the increased thrombogenic potential of human atherosclerotic plaques and may be an important determinant of plaque thrombosis upon plaque rupture. In addition, we have recently found that a significant percentage of luminal endothelial cells may undergo apoptosis in human atherosclerotic plaques (Tricot *et al.*, 2000). We believe that these apoptotic endothelial cells may initiate plaque erosion and promote local platelet aggregation and thrombosis *in vivo*, being therefore responsible for acute ischaemic syndromes. Finally, experiments in our laboratory show that plasma levels of procoagulant microparticles are increased in patients with acute coronary syndromes and may be associated with coronary reocclusion (Mallat *et al.*, 2000).

#### *Therapeutic modulation of apoptosis in atherosclerosis*

Given the importance of apoptosis in plaque development and stability, interventions designed to modulate the apoptotic process are warranted and may profoundly affect disease evolution.

*Induction of apoptosis to promote plaque regression* (Table 4) Pollman *et al.* (1998) have reported regression of atherosclerotic plaques of cholesterol-fed rabbits after inhibition of neointimal cell Bcl-x expression and induction of neointimal cell apoptosis. Wang *et al.* (1999) have also observed regression of rabbit atheromatous lesions following *in vivo* administration of L-arginine to induce macrophage apoptosis *via* NO release. However, it is still unknown whether human atherosclerotic plaques will behave in the same manner. Rabbit atherosclerotic plaques do not rupture whereas human plaques are prone to rupture. In addition, others did not confirm this finding by using a different approach to induce intimal cell apoptosis (overexpression of FADD) (Schaub *et al.*, 1998). Rather, vascular inflammation followed the induction of the apoptotic process and led to neointima progression (Schaub *et al.*, 1998). On the other hand, it could be hazardous to induce massive apoptotic cell death in human atherosclerotic plaques without ensuring high anti-coagulation levels given the increased procoagulant potential of apoptotic cells (Bombeli *et al.*, 1997; Flynn *et al.*, 1997; Mallat *et al.*, 1999b).

Plaque neovascularization may be involved in plaque development and progression (Moulton *et al.*, 1999). Therefore, induction of endothelial cell apoptosis within plaque neovessels, using angiostatin or endostatin for example, could be a potential approach to limit plaque progression. However, such therapy would also induce apoptotic death in luminal endothelial cells with the potential of inducing a procoagulant state at the interface between the plaque and the circulating blood.

**Table 4** Potential effects of induction of apoptosis in atherosclerosis

<i>Type of intervention</i>	<i>Expected beneficial effects</i>	<i>Expected deleterious effects</i>
<ul style="list-style-type: none"> <li>Overexpression of pro-apoptotic genes (*Bcl-x, FADD, FasL...)</li> </ul>	<ul style="list-style-type: none"> <li>Cell apoptosis → neointima regression</li> </ul>	<ul style="list-style-type: none"> <li>Pro-inflammatory reaction if massive apoptosis → neointima progression.</li> <li>Procoagulant potential of apoptotic cells and particles → increased plaque thrombogenicity.</li> <li>Weakening of fibrous cap (death of VSMCs)</li> </ul>
<ul style="list-style-type: none"> <li>Administration of NO (L-arginine, transfer of NOS II...)</li> </ul>	<ul style="list-style-type: none"> <li>Macrophage apoptosis → lesion regression</li> </ul>	<ul style="list-style-type: none"> <li>Accumulation of lipids and apoptotic particles → increased lipid core dimension and thrombogenicity</li> </ul>
<ul style="list-style-type: none"> <li>Angiostatin, endostatin administration</li> </ul>	<ul style="list-style-type: none"> <li>Endothelial cell apoptosis, inhibition of angiogenesis → plaque regression</li> </ul>	<ul style="list-style-type: none"> <li>Luminal endothelial cell apoptosis → Plaque erosion and thrombosis</li> </ul>

**Table 5** Potential effects of inhibition of apoptosis in atherosclerosis

<i>Type of intervention</i>	<i>Expected beneficial effects</i>	<i>Expected side effects</i>
<ul style="list-style-type: none"> <li>Administration of NO (transfer of NOS III...)</li> </ul>	<ul style="list-style-type: none"> <li>Inhibition of endothelial apoptosis → prevention of plaque erosion/thrombosis</li> </ul>	<ul style="list-style-type: none"> <li>Macrophage apoptosis?</li> </ul>
<ul style="list-style-type: none"> <li>Administration of endothelial survival factors (VEGF, FGF-2...)</li> </ul>	<ul style="list-style-type: none"> <li>Inhibition of endothelial apoptosis → prevention of plaque erosion/thrombosis</li> </ul>	<ul style="list-style-type: none"> <li>Neoangiogenesis → plaque progression/rupture</li> </ul>
<ul style="list-style-type: none"> <li>Estrogen treatment</li> </ul>	<ul style="list-style-type: none"> <li>Inhibition of endothelial apoptosis → Prevention of plaque erosion/thrombosis</li> </ul>	
<ul style="list-style-type: none"> <li>Angiotensin converting enzyme inhibitors</li> </ul>	<ul style="list-style-type: none"> <li>Inhibition of endothelial apoptosis → Prevention of plaque erosion/thrombosis</li> </ul>	
<ul style="list-style-type: none"> <li>Anti-oxidant therapy (HO-1 overexpression, vitamins)</li> </ul>	<ul style="list-style-type: none"> <li>Inhibition of cell apoptosis → decreased risk of plaque disruption thrombosis</li> </ul>	
<ul style="list-style-type: none"> <li>Anti-inflammatory therapy (e.g., IL-10)</li> </ul>	<ul style="list-style-type: none"> <li>Decreased inflammation → inhibition of apoptosis and thrombosis</li> </ul>	

*Inhibition of apoptosis to prevent plaque rupture/erosion and thrombosis (Table 5)* We have shown that apoptosis occurs in the different cell types present in human atherosclerotic plaques, including endothelial cells, and may therefore initiate plaque disruption and thrombosis (Mallat *et al.*, 1999b; Tricot *et al.*, 2000). We believe that strategies aimed at the inhibition of apoptosis may limit plaque erosion, thrombosis and progression. Nitric oxide is a powerful anti-apoptotic molecule for endothelial cells (Dimmeler *et al.*, 1997b) and accounts, at least in part, for the anti-apoptotic effects of laminar shear stress on endothelial cells *in vitro*. *In vivo*, the apoptotic luminal endothelial cells present in human atherosclerotic plaques are preferentially located in the low shear stress post-stenotic area (Tricot *et al.*, 2000) where NO production can be expected to be reduced. Therefore, *in vivo* delivery of NO, *via* supplementation with L-arginine or transfer of the NOS III gene for example, or *in vivo* delivery of endothelial survival factors such as VEGF or FGF-2, may limit luminal endothelial cell apoptosis and thrombosis, and alter plaque progression.

*In vitro* studies have shown that angiotensin II induces endothelial cell apoptosis (Dimmeler *et al.*, 1997c; Li *et al.*, 1999a,b). The apoptotic process is prevented by addition of NO donors or angiotensin II antagonists (Dimmeler *et al.*, 1997c; Li *et al.*, 1999a,b). One could be argued that *in vivo* inhibition of angiotensin II production or signalling may prevent endothelial cell apoptosis. Recently, angiotensin-converting enzyme inhibitors have been shown to significantly reduce the occurrence of myocardial infarction in humans (Yusuf *et al.*, 2000). We speculate that a reduction in endothelial cell apoptosis and thrombosis is one of the potential mechanisms responsible for this beneficial effect.

Oestrogen has been shown to be anti-atherogenic and to exert beneficial effects in the regulation of vascular tone in atherosclerotic arteries. Oestradiol treatment of HUVEC in culture results in a dose-dependent, receptor-mediated inhibition of TNF- $\alpha$ -induced apoptosis and activation of the caspase-1 pathway (Spyridopoulos *et al.*, 1997). Moreover, oestrogen administration increases the release of bioactive nitric oxide by inhibiting superoxide anion production (Arnal *et al.*, 1996). These data suggest that the beneficial atheroprotective effects of oestradiol may result, at least in part, from the preservation of endothelial integrity and prevention of vessel thrombosis. This may also be the case of vitamins with anti-oxidant properties which inhibit oxidized LDL-induced endothelial cell apoptosis *in vitro* (Dimmeler *et al.*, 1997a).

Another powerful anti-oxidant and anti-apoptotic product for endothelial cells is the product of the haeme oxygenase (HO)-1 gene (Ferris *et al.*, 1999; Hancock *et al.*, 1998; Yachie *et al.*, 1999). Deficiency in HO-1 is associated with severe endothelial damage (Ferris *et al.*, 1999; Hancock *et al.*, 1998), while overexpression of HO-1 is associated with expression of various cytoprotective genes, such as Bcl-2, and prevents from transplant arteriosclerosis (Hancock *et al.*, 1998). Moreover, endothelial integrity in this setting is associated with the expression of anti-inflammatory cytokines including IL-10 (Bach *et al.*, 1997).

Finally, occurrence of apoptosis within human atherosclerotic plaques is highly dependent on the inflammatory balance (Mallat *et al.*, 1999a). Expression of the anti-inflammatory cytokine IL-10 within the plaque is associated with decreased signs of inflammation and decreased apoptosis (Mallat *et al.*, 1999a). Moreover, IL-10 plays an important protective role against the development of atherosclerosis in mice (Mallat *et al.*, 1999c), and displays potential anti-thrombotic effects *in vivo* (Downing *et al.*, 1998; Ernoffsson *et al.*, 1996). Therefore, *in vivo* administration of IL-10 might be a

sound strategy to deactivate inflammatory cells, reduce apoptotic death and its pro-thrombogenic properties, leading to plaque stabilization. These effects may be beneficial in both atherosclerosis and restenosis.

### *Apoptosis in restenosis*

Animal models of neointima formation following balloon injury have shown that apoptotic cell death occurs at different points in time and at variable levels during the repair process. Apoptotic death of smooth muscle cells is observed as early as 30 min after arterial injury and is associated with decreased expression of the anti-apoptotic protein Bcl-x (Perlman *et al.*, 1997). After then, a second window of apoptosis is observed and is associated with increased smooth muscle cell proliferation (Han *et al.*, 1995). Persistent apoptotic cell death at later stages, in the absence of cell proliferation, has been implicated in the regulation of intimal thickening (Bochaton-Piallat *et al.*, 1995).

Studies available in human restenotic plaques are somewhat contradictory. Isner *et al.* observed an increased apoptotic rate in restenotic versus primary coronary plaques (Isner *et al.*, 1995). However, this finding was recently challenged by Bauriedel *et al.* (1998) who observed decreased levels of apoptosis in restenotic versus primary plaques with no differences in necrosis levels. High cellularity is a key finding in late human restenosis and the findings of Bauriedel *et al.* (1998) are more consistent with the paradigm that lower rates of apoptosis result in hyperplasia. This also fits well with the finding of decreased p53 activity (and potentially p53-mediated apoptosis) in restenotic material from human coronary lesions (Speir *et al.*, 1994).

The observation that apoptosis may participate in cellularity regulation in animal models of neointima formation and in human restenotic lesions prompted researchers to examine the effects of pro-apoptotic strategies on this process. Steg *et al.* (1997) used suicide gene therapy by transfer of a replication-defective adenoviral vector expressing the thymidine kinase gene combined with ganciclovir treatment. *In vitro* transfection and treatment of SMCs induced their death by apoptosis, and this mechanism was presumably responsible for the reduction of restenosis after angioplasty in transfected and treated atheromatous rabbit arteries. Pollman *et al.* (1998) used another approach interfering with the anti-apoptotic genes expressed in the neointima, which consisted in the local application of antisense oligonucleotides encoding a sequence complementary to the coding region for the anti-apoptotic *bcl-x* gene. Using this strategy, they were able to decrease neointimal expression of Bcl-x, inducing selective apoptosis of neointimal cells and regression of neointimal hyperplasia. Other investigators have used a direct pro-apoptotic strategy delivering Fas ligand into the vessel wall and inducing apoptosis of Fas-bearing VSMCs (Sata *et al.*, 1998). This also led to significant inhibition of neointima formation adding to the evidence that apoptosis is a key determinant of neointimal thickening evolution after arterial injury. However, it remains to be determined whether such impressive results may be achieved in humans without serious side effects, namely vessel inflammation and thrombosis.

## Conclusion

The observation that cell death and cell proliferation may occur independently and the growing understanding of the molecular basis for these phenomena will stimulate the development of interventions selectively aimed at cell death to prevent the progression of a variety of vessel diseases.

## References

- ALON, T., HEMO, I., ITIN, A., PE'ER, J., STONE, J. & KESHET, E. (1995). Vascular endothelial growth factor acts as a survival factor for newly formed retinal vessels and has implications for retinopathy of prematurity. *Nat. Med.*, **1**, 1024–1028.
- ALVAREZ, R.J., GIPS, S.J., MOLDOVAN, N., WILHIDE, C.C., MILLIKEN, E.E., HOANG, A.T., HRUBAN, R.H., SILVERMAN, H.S., DANG, C.V. & GOLDSCHMIDT-CLERMONT, P.J. (1997).  $17\beta$ -estradiol inhibits apoptosis of endothelial cells. *Biochem. Biophys. Res. Commun.*, **237**, 372–381.
- ARNAL, J.F., CLAMENS, S., PECHET, C., NEGRESALVAYRE, A., ALLERA, C., GIROLAMI, J.P., SALVAYRE, R. & BAYARD, F. (1996). Ethinylestradiol does not enhance the expression of nitric oxide synthase in bovine endothelial cells but increases the release of bioactive nitric oxide by inhibiting superoxide anion production. *Proc. Natl. Acad. Sci. U.S.A.*, **93**, 4108–4113.
- ASHKENAZI, A. & DIXIT, V.M. (1998). Death receptors: signaling and modulation. *Science*, **281**, 1305–1308.
- AUPEIX, K., HUGEL, B., MARTIN, T., BISCHOFF, P., LILL, H., PASQUALI, J.L. & FREYSSINET, J.M. (1997). The significance of shed membrane particles during programmed cell death in vitro, and in vivo, in HIV-1 infection. *J. Clin. Invest.*, **99**, 1546–1554.
- BACH, F.H., FERRAN, C., HECHENLEITNER, P., MARK, W., KOYAMADA, N., MIYATAKE, T., WINKLER, H., BADRICHANI, A., CANDINAS, D. & HANCOCK, W.W. (1997). Accommodation of vascularized xenografts: Expression of 'protective genes' by donor endothelial cells in a host Th2 cytokine environment. *Nature Med.*, **3**, 196–204.
- BACH, R., GENTRY, R. & NEMERSON, Y. (1986). Factor VII binding to tissue factor in reconstituted phospholipid vesicles: induction of cooperativity by phosphatidylserine. *Biochemistry*, **25**, 4007–4020.
- BADRICHANI, A.Z., STROKA, D.M., BILBAO, G., CURIEL, D.T., BACH, F.H. & FERRAN, C. (1999). Bcl-2 and Bcl-X<sub>L</sub> serve an anti-inflammatory function in endothelial cells through inhibition of NF- $\kappa$ B. *J. Clin. Invest.*, **103**, 543–553.
- BAI, H., POLLMAN, M.J., INISHI, Y. & GIBBONS, G.H. (1999). Regulation of vascular smooth muscle cell apoptosis. Modulation of bad by a phosphatidylinositol 3-kinase-dependent pathway. *Circ. Res.*, **85**, 229–237.
- BAKER, A.H., ZALTSMAN, A.B., GEORGE, S.J. & NEWBY, A.C. (1998). Divergent effects of tissue inhibitor of metalloproteinase-1, -2 or -3 overexpression on rat vascular smooth muscle cell invasion, proliferation, and death in vitro. *J. Clin. Invest.*, **101**, 1478–1487.
- BAURIEDL, G., SCHLUCKEBIER, S., HUTTER, R., WELSCH, U., KANDOLF, R., LÜDERITZ, B. & PRESCOTT, M.F. (1998). Apoptosis in restenosis versus stable-angina atherosclerosis. Implications for the pathogenesis of restenosis. *Arterioscler. Thromb. Vasc. Biol.*, **18**, 1132–1139.
- BENNETT, M.R., EVAN, G.I. & NEWBY, A.C. (1994). Deregulated expression of the c-myc oncogene abolishes inhibition of proliferation of rat vascular smooth muscle cells by serum reduction, interferon- $\gamma$ , heparin, and cyclic nucleotide analogues and induces apoptosis. *Circ. Res.*, **74**, 525–536.
- BENNETT, M.R., EVAN, G.I. & SCHWARTZ, S.M. (1995a). Apoptosis of rat vascular smooth muscle cells is regulated by p53-dependent and -independent pathways. *Circ. Res.*, **77**, 266–273.
- BENNETT, M.R., EVAN, G.I. & SCHWARTZ, S.M. (1995b). Apoptosis of human vascular smooth muscle cells derived from normal vessels and coronary atherosclerotic plaques. *J. Clin. Invest.*, **95**, 2266–2274.
- BENNETT, M.R., LITTLEWOOD, T.D., SCHWARTZ, S.M. & WEISSBERG, P.L. (1997). Increased sensitivity of human vascular smooth muscle cells from atherosclerotic plaques to p53-mediated apoptosis. *Circ. Res.*, **81**, 591–599.
- BENNETT, M.R., MACDONALD, K., CHAN, S.W., BOYLE, J.J. & WEISSBERG, P.L. (1998a). Cooperative interactions between RB and p53 regulate cell proliferation, cell senescence, and apoptosis in human vascular smooth muscle cells from atherosclerotic plaques. *Circ. Res.*, **82**, 704–712.
- BENNETT, M.R., MACDONALD, K., CHAN, S.W., LUZIO, J.P., SIMARI, R. & WEISSBERG, P. (1998b). Cell surface trafficking of Fas: a rapid mechanism of p53-mediated apoptosis. *Science*, **282**, 290–293.
- BIRD, D.A., GILLOTTE, K.L., HORKKO, S., FRIEDMAN, P., DENNIS, E.A., WITZTUM, J.L. & STEINBERG, D. (1999). Receptors for oxidized low-density lipoprotein on elicited mouse peritoneal macrophages can recognize both the modified lipid moieties and the modified protein moieties: implications with respect to macrophage recognition of apoptotic cells. *Proc. Natl. Acad. Sci. U.S.A.*, **96**, 6347–6352.
- BJÖRKERUD, S. & BJÖRKERUD, B. (1996). Apoptosis is abundant in human atherosclerotic lesions, especially in inflammatory cells (macrophages and T cells), and may contribute to the accumulation of gruel and plaque instability. *Am. J. Pathol.*, **149**, 367–380.
- BOCHATON-PIALLAT, M.L., GABBIANI, F., REDARD, M., DESMOULIÈRE, A. & GABBIANI, G. (1995). Apoptosis participates in cellularly regulation during rat aortic intimal thickening. *Am. J. Pathol.*, **146**, 1059–1064.
- BOMBELI, T., KARSAN, A., TAIT, J.F. & HARLAN, J.M. (1997). Apoptotic vascular endothelial cells become procoagulant. *Blood*, **89**, 2429–2442.
- BOYLE, J.J., BOWYER, D.E., PROUDFOOT, D., WEISSBERG, P.L. & BENNETT, M.R. (1998). Human monocyte/macrophages induce human vascular smooth muscle cell apoptosis in culture. *Circulation*, **98**, I-598. Abstract.
- BRANDL, R., RICHTER, T., HAUG, K., WILHELM, M.G., MAURER, P.C. & NATHRATH, W. (1997). Topographic analysis of proliferative activity in carotid endarterectomy specimens by immunocytochemical detection of the cell cycle-related antigen Ki-67. *Circulation*, **96**, 3360–3368.
- BROOKS, P.C., MONTGOMERY, A.M.P., ROSENFELD, M., REISFELD, R.A., HU, T.H., KLIER, G. & CHERESH, D.A. (1994). Integrin  $\alpha_v\beta_3$  antagonists promote tumor regression by inducing apoptosis of angiogenic blood vessels. *Cell*, **79**, 1157–1164.
- CAI, W., DEVAUX, B., SCHAPER, W. & SCHAPER, J. (1997). The role of Fas/APO 1 and apoptosis in the development of human atherosclerotic lesions. *Atherosclerosis*, **131**, 177–186.
- CARMELIET, P., LAMPUGNANI, M.G., MOONS, L., BREVIARIO, F., COMPERNOLLE, V., BONO, F., BALCONI, G., SPAGNUOLO, R., OOSTUYSE, B., DEWERCHIN, M., ZANETTI, A., ANGELLILO, A., MATTOT, V., NUYENS, D., LUTGENS, E., CLOTMAN, F., DE RUITER, M.C., GITTENBERGER-DE-GROOT, A., POELMANN, R., LUPU, F., HERBERT, J.M., COLLEN, D. & DEJANA, E. (1999). Targeted deficiency or cytosolic truncation of the VE-cadherin gene in mice impairs VEGF-mediated endothelial survival and angiogenesis. *Cell*, **98**, 147–157.
- CASCIOLA-ROSEN, L.A., ANHALT, G. & ROSEN, A. (1994). Autoantigens targeted in systemic lupus erythematosus are clustered in two populations of surface structures on apoptotic keratinocytes. *J. Exp. Med.*, **179**, 1317–1330.
- CHANG, M.K., BERGMARK, C., LAURILA, A., HORKKO, S., HAN, K.H., FRIEDMAN, P., DENNIS, E.A. & WITZTUM, J.L. (1999). Monoclonal antibodies against oxidized low-density lipoprotein bind to apoptotic cells and inhibit their phagocytosis by elicited macrophages: evidence that oxidation-specific epitopes mediate macrophage recognition. *Proc. Natl. Acad. Sci. U.S.A.*, **96**, 6353–6358.
- CHO, A., COURTMAN, D.W. & LANGILLE, B.L. (1995). Apoptosis (programmed cell death) in arteries of the neonatal lamb. *Circ. Res.*, **76**, 168–175.
- CLAESSON-WELSH, L., WELSH, M., ITO, N., ANAND-APTE, B., SOKER, S., ZETTER, B., O'REILLY, M. & FOLKMAN, J. (1998). Angiostatin induces endothelial cell apoptosis and activation of focal adhesion kinase independently of the integrin-binding motif RGD. *Proc. Natl. Acad. Sci. U.S.A.*, **95**, 5579–5583.
- CLIFF, W.J. (1970). The aortic tunica media in aging rats. *Exp. Mol. Pathol.*, **13**, 172–189.
- COLEMAN, K.R., BRADEN, G.A., WILLINGHAM, M.C. & SANE, D.C. (1999). Vitaxin, a humanized monoclonal antibody to the vitronectin receptor ( $\alpha_v\beta_3$ ), reduces neointimal hyperplasia and total vessel area after balloon injury in hypercholesterolemic rabbits. *Circ. Res.*, **84**, 1268–1276.
- DEVITT, A., MOFFATT, O.D., RAYKUNDALIA, C., CAPRA, J.D., SIMMONS, D.L. & GREGORY, C.D. (1998). Human CD14 mediates recognition and phagocytosis of apoptotic cells. *Nature*, **392**, 505–509.

- DIEZ, J., FORTUNO, M.A. & RAVASSA, S. (1998). Apoptosis in hypertensive heart disease. *Curr. Opin. Cardiol.*, **13**, 317–325.
- DIMMELER, S., ASSMUS, B., HERMANN, C., HAENDELER, J. & ZEIHNER, A.M. (1998). Fluid shear stress stimulates phosphorylation of Akt in human endothelial cells: involvement in suppression of apoptosis. *Circ. Res.*, **83**, 334–341.
- DIMMELER, S., BREITSCHOPF, K., HAENDELER, J. & ZEIHNER, A.M. (1999b). Dephosphorylation targets Bcl-2 for ubiquitin-dependent degradation: a link between the apoptosome and the proteasome pathway. *J. Exp. Med.*, **189**, 1815–1822.
- DIMMELER, S., FLEMING, I., FISSLTHALER, B., HERMANN, C., BUSSE, R. & ZEIHNER, A.M. (1999a). Activation of nitric oxide synthase in endothelial cells by Akt-dependent phosphorylation. *Nature*, **399**, 601–605.
- DIMMELER, S., HAENDELER, J., GALLE, J. & ZEIHNER, A.M. (1997a). Oxidized low-density lipoprotein induces apoptosis of human endothelial cells by a link between the CPP32-like proteases: A mechanistic clue to the 'response to injury' hypothesis. *Circulation*, **95**, 1760–1763.
- DIMMELER, S., HAENDELER, J., NEHLS, M. & ZEIHNER, A.M. (1997b). Suppression of apoptosis by nitric oxide via inhibition of interleukin-1 beta-converting enzyme (ICE)-like and cysteine protease protein (CPP)-32-like proteases. *J. Exp. Med.*, **185**, 601–607.
- DIMMELER, S., HAENDELER, J., RIPPIMANN, V., NEHLS, M. & ZEIHNER, A.M. (1996). Shear stress inhibits apoptosis of human endothelial cells. *FEBS Lett.*, **399**, 71–74.
- DIMMELER, S., RIPPIMANN, V., WEILAND, U., HAENDELER, J. & ZEIHNER, A.M. (1997c). Angiotensin II induces apoptosis of human endothelial cells. Protective effect of nitric oxide. *Circ. Res.*, **81**, 970–976.
- DIMMELER, S. & ZEIHNER, A.M. (1999). Nitric oxide—an endothelial cell survival factor. *Cell Death Differ.*, **6**, 964–968.
- DONG, C., WILSON, J.E., WINTERS, G.L. & MCMANUS, B.M. (1996). Human transplant coronary artery disease: Pathological evidence for Fas-mediated apoptotic cytotoxicity in allograft arteriopathy. *Lab. Invest.*, **74**, 921–931.
- DOWNING, L.J., STRIETER, R.M., KADELL, A.M., WILKE, C.A., AUSTIN, J.C., HARE, B.D., BURDICK, M.D., GREENFIELD, L.J. & WAKEFIELD, T.W. (1998). IL-10 regulates thrombus-induced vein wall inflammation and thrombosis. *J. Immunol.*, **161**, 1471–1476.
- DUFOURCQ, P., LOUIS, H., MOREAU, C., DARET, D., BOISSEAU, M.R., LAMAZIERE, J.M. & BONNET, J. (1998). Vitronectin expression and interaction with receptors in smooth muscle cells from human atheromatous plaque. *Arterioscler. Thromb. Vasc. Biol.*, **18**, 168–176.
- ENARI, M., SAKAHIRA, H., YOKOYAMA, H., OKAWA, K., IWAMATSU, A. & NAGATA, S. (1998). A caspase-activated DNase that degrades DNA during apoptosis, and its inhibitor ICAD. *Nature*, **391**, 43–50.
- ERNOFSSON, M., TENNO, T. & SIEGBAHN, A. (1996). Inhibition of tissue factor surface expression in human peripheral blood monocytes exposed to cytokines. *Br. J. Haematol.*, **95**, 249–257.
- ESCARQUEIL-BLANC, I., MEILHAC, O., PIERAGGI, M.-T., ARNAL, J.-F., SALVAYRE, R. & NÈGRE-SALVAYRE, A. (1997). Oxidized LDLs induce massive apoptosis of cultured human endothelial cells through a calcium-dependent pathway: prevention by aurointricarboxylic acid. *Arterioscler. Thromb. Vasc. Biol.*, **17**, 331–339.
- EVAN, G.I., WYLLIE, A.H., GILBERT, C.S., LITTLEWOOD, T.D., LAND, H., BROOKS, M., WATERS, C.M., PENN, L.Z. & HANCOCK, D.C. (1992). Induction of apoptosis in fibroblasts by c-myc protein. *Cell*, **69**, 119–128.
- FADOK, V.A., SAVILL, J.S., HASLETT, C., BRATTON, D.L., DOHERTY, D.E., CAMPBELL, P.A. & HENSON, P.M. (1992). Different populations of macrophages use either the vitronectin receptor or the phosphatidylserine receptor to recognize and remove apoptotic cells. *J. Immunol.*, **149**, 4029–4035.
- FADOK, V.A., WARNER, M.L., BRATTON, D.L. & HENSON, P.M. (1998). CD36 is required for phagocytosis of apoptotic cells by human macrophages that use either a phosphatidylserine receptor or the vitronectin receptor ( $\alpha_v\beta_3$ ). *J. Immunol.*, **161**, 6250–6257.
- FERRIS, C.D., JAFFREY, S.R., SAWA, A., TAKAHASHI, M., BRADY, S.D., BARROW, R.K., TYSOE, S.A., WOLOSKE, H., BARANANO, D.E., DORE, S., POSS, K.D. & SNYDER, S.H. (1999). Haem oxygenase-1 prevents cell death by regulating cellular iron. *Nat. Cell. Biol.*, **1**, 152–157.
- FLYNN, P.D., BYRNE, C.D., BAGLIN, T.P., WEISSBERG, P.L. & BENNETT, M.R. (1997). Thrombin generation by apoptotic vascular smooth muscle cells. *Blood*, **89**, 4378–4384.
- FOX, J.C. & SHANLEY, J.R. (1996). Antisense inhibition of basic fibroblast growth factor induces apoptosis in vascular smooth muscle cells. *J. Biol. Chem.*, **271**, 12578–12584.
- FUJIKAWA, K., DE AOS SCHERPENSEEL, I., JAIN, S.K., PRESMAN, E. & VARTICOVSKI, L. (1999). Role of PI 3-kinase in angiopoietin-1-mediated migration and attachment-dependent survival of endothelial cells [In Process Citation]. *Exp. Cell. Res.*, **253**, 663–672.
- GALIS, Z.S., SUKHOVA, G.K., LARK, M.W. & LIBBY, P. (1994). Increased expression of matrix metalloproteinases and matrix degrading activity in vulnerable regions of human atherosclerotic plaques. *J. Clin. Invest.*, **94**, 2493–2503.
- GENG, Y.J., HENDERSON, L.E., LEVESQUE, E.B., MUSZYNSKI, M. & LIBBY, P. (1997). Fas is expressed in human atherosclerotic intima and promotes apoptosis of cytokine-primed human vascular smooth muscle cells. *Arterioscler. Thromb. Vasc. Biol.*, **17**, 2200–2208.
- GENG, Y.-J. & LIBBY, P. (1995). Evidence for apoptosis in advanced human atheroma. Colocalization with interleukin-1 $\beta$ -converting enzyme. *Am. J. Pathol.*, **147**, 251–266.
- GENG, Y.J., WU, Q., MUSZYNSKI, M., HANSSON, G.K. & LIBBY, P. (1996). Apoptosis of vascular smooth muscle cells induced by in vitro stimulation with interferon-gamma, tumor necrosis factor-alpha, and interleukin-1 beta. *Arterioscler. Thromb. Vasc. Biol.*, **16**, 19–27.
- GERBER, H.P., DIXIT, V. & FERRARA, N. (1998). Vascular endothelial growth factor induces expression of the antiapoptotic proteins Bcl-2 and A1 in vascular endothelial cells. *J. Biol. Chem.*, **273**, 13313–13316.
- GIDON-JEANGIRARD, C., HUGEL, B., HOLL, V., TOTI, F., LAPLANCHE, J.L., MEYER, D. & FREYSSINET, J.M. (1999). Annexin V delays apoptosis while exerting an external constraint preventing the release of CD4+ and PrPc+ membrane particles in a human T lymphocyte model. *J. Immunol.*, **162**, 5712–5718.
- GOLSTEIN, P. (1997). Controlling cell death. *Science*, **275**, 1081–1082.
- GOPING, I.S., GROSS, A., LAVOIE, J.N., NGUYEN, M., JEMMERSON, R., ROTH, K., KORSMEYER, S.J. & SHORE, G.C. (1998). Regulated targeting of BAX to mitochondria. *J. Cell Biol.*, **143**, 207–215.
- GORDON, D., REIDY, M.A., BENDITT, E.P. & SCHWARTZ, S.M. (1990). Cell proliferation in human coronary arteries. *Proc. Natl. Acad. Sci. U.S.A.*, **87**, 4600–4604.
- GREEN, D. & KROEMER, G. (1998). The central executioners of apoptosis: caspases or mitochondria? *Trends Cell. Biol.*, **8**, 267–271.
- GREEN, D.R. & REED, J.C. (1998). Mitochondria and apoptosis. *Science*, **281**, 1309–1311.
- GRIFFITHS, G.J., DUBREZ, L., MORGAN, C.P., JONES, N.A., WHITEHOUSE, J., CORFE, B.M., DIVE, C. & HICKMAN, J.A. (1999). Cell damage-induced conformational changes of the pro-apoptotic protein Bak in vivo precede the onset of apoptosis. *J. Cell Biol.*, **144**, 903–914.
- HAENDELER, J., ZEIHNER, A.M. & DIMMELER, S. (1996). Vitamin C and E prevent lipopolysaccharide-induced apoptosis in human endothelial cells by modulation of Bcl-2 and Bax. *Eur. J. Pharmacol.*, **317**, 407–411.
- HAIMOVITZ-FRIEDMAN, A., CORDON-CARDO, C., BAYOUMY, S., GARZOTTO, M., MCLOUGHLIN, M., GALLILY, R., EDWARDS, III, C.K., SCHUCHMAN, E.H., FUKS, Z. & KOLESNICK, R. (1997). Lipopolysaccharide induces disseminated endothelial apoptosis requiring ceramide generation. *J. Exp. Med.*, **186**, 1831–1841.
- HAMET, P., MOREAU, P., DAM, T.V., ORLOV, S.N., TEA, B.S., DEBLOIS, D. & TREMBLAY, J. (1996). The time window of apoptosis: A new component in the therapeutic strategy for cardiovascular remodeling. *J. Hypertens.*, **14**, S65–S70.
- HAN, D.K.M., HAUDENSCHILD, C.C., HONG, M.K., TINKLE, B.T., LEON, M.B. & LIAU, G. (1995). Evidence for apoptosis in human atherogenesis and in a rat vascular injury model. *Am. J. Pathol.*, **147**, 267–277.
- HAN, D.K.M., WRIGHT, M.E., DIXIT, V.M., PRUIT, R., SON SOE, M., LYNCH, D.H. & SCHWARTZ, S.M. (1996). Evidence for escape of apoptosis by a loss of Fas in atherosclerotic plaque smooth muscle cells. *Circulation*, **94** (suppl I), I-397.
- HANCOCK, W.W., BUELOW, R., SAYEGH, M.H. & TURKA, L.A. (1998). Antibody-induced transplant arteriosclerosis is prevented by graft expression of anti-oxidant and anti-apoptotic genes. *Nat. Med.*, **4**, 1392–1396.

- HARADA-SHIBA, M., KINOSHITA, M., KAMIDO, H. & SHIMOKADO, K. (1998). Oxidized low density lipoprotein induces apoptosis in cultured human umbilical vein endothelial cells by common and unique mechanisms. *J. Biol. Chem.*, **273**, 9681–9687.
- HAUNSTETTER, A. & IZUMO, S. (1998). Apoptosis: basic mechanisms and implications for cardiovascular disease. *Circ. Res.*, **82**, 1111–1129.
- HAYES, A.J., HUANG, W.Q., MALLAH, J., YANG, D., LIPPMAN, M.E. & LI, L.Y. (1999). Angiopoietin-1 and its receptor Tie-2 participate in the regulation of capillary-like tubule formation and survival of endothelial cells. *Microvasc. Res.*, **58**, 224–237.
- HEGYI, L., SKEPPER, J.N., CARY, N.R. & MITCHINSON, M.J. (1996). Foam cell apoptosis and the development of the lipid core of human atherosclerosis. *J. Pathol.*, **180**, 423–442.
- HERMANN, C., ZEIHNER, A.M. & DIMMELER, S. (1997). Shear stress inhibits H<sub>2</sub>O<sub>2</sub>-induced apoptosis of human endothelial cells by modulation of the glutathione redox cycle and nitric oxide synthase. *Arterioscler. Thromb. Vasc. Biol.*, **17**, 3588–3592.
- HERREN, B., LEVKAU, B., RAINES, E.W. & ROSS, R. (1998). Cleavage of beta-catenin and plakoglobin and shedding of VE-cadherin during endothelial apoptosis: evidence for a role for caspases and metalloproteinases. *Mol. Biol. Cell*, **9**, 1589–1601.
- HESSLER, J.L., ROBERTSON, A.L. & CHISOLM, G.M. (1979). LDL-induced cytotoxicity and its inhibition by HDL in human vascular smooth muscle and endothelial cells in culture. *Atherosclerosis*, **32**, 213–229.
- HETTS, S.W. (1998). To die or not to die: an overview of apoptosis and its role in disease. *JAMA*, **279**, 300–307.
- HOLASH, J., MAISONPIERRE, P.C., COMPTON, D., BOLAND, P., ALEXANDER, C.R., ZAGZAG, D., YANCOPOULOS, G.D. & WIEGAND, S.J. (1999). Vessel cooption, regression, and growth in tumors mediated by angiopoietins and VEGF. *Science*, **284**, 1994–1998.
- HOWARD, M., MUCHAMEL, T., ANDRADE, S. & MENON, S. (1993). Interleukin 10 protects mice from lethal endotoxemia. *J. Exp. Med.*, **177**, 1205–1208.
- ILIC, D., ALMEIDA, E.A., SCHLAEPFER, D.D., DAZIN, P., AIZAWA, S. & DAMSKY, C.H. (1998). Extracellular matrix survival signals transduced by focal adhesion kinase suppress p53-mediated apoptosis. *J. Cell. Biol.*, **143**, 547–560.
- ISNER, J.M., KEARNEY, M., BORTMAN, S. & PASSERI, J. (1995). Apoptosis in human atherosclerosis and restenosis. *Circulation*, **91**, 2703–2711.
- JONES, P.L., COWAN, K.N. & RABINOVITCH, M. (1997b). Tenascin-C, proliferation and subendothelial fibronectin in progressive pulmonary vascular disease. *Am. J. Pathol.*, **150**, 1349–1360.
- JONES, P.L., CRACK, J. & RABINOVITCH, M. (1997a). Regulation of tenascin-C, a vascular smooth muscle cell survival factor that interacts with the  $\alpha_5\beta_3$  integrin to promote epidermal growth factor receptor phosphorylation and growth. *J. Cell Biol.*, **139**, 279–293.
- JORINGE, S., CRISBY, M., THYBERG, J. & NILSSON, J. (1997). DNA fragmentation and ultrastructural changes of degenerating cells in atherosclerotic lesions and smooth muscle cells exposed to oxidized LDL in vitro. *Arterioscler. Thromb. Vasc. Biol.*, **17**, 2225–2231.
- KAISER, D., FREYBERG, M.A. & FRIEDL, P. (1997). Lack of hemodynamic forces triggers apoptosis in vascular endothelial cells. *Biochem. Biophys. Res. Commun.*, **231**, 586–590.
- KARSAN, A. (1998). Tumor necrosis factor and endothelial cell death. *Trends Cardiovasc. Med.*, **8**, 19–24.
- KARSAN, A., YEE, E., KAUSHANSKY, K. & HARLAN, J.M. (1996). Cloning of a human Bcl-2 homologue: inflammatory cytokines induce human A1 in cultured endothelial cells. *Blood*, **87**, 3089–3096.
- KARSAN, A., YEE, E., POIRIER, G.G., ZHOU, P., CRAIG, R. & HARLAN, J.M. (1997). Fibroblast growth factor-2 inhibits endothelial cell apoptosis by Bcl-2 dependent and independent mechanisms. *Am. J. Pathol.*, **151**, 1775–1784.
- KATO, O., TAUCHI, H., KAWAISHI, K., KIMURA, A. & SATOW, Y. (1995). Expression of the vascular endothelial growth factor (VEGF) receptor gene, KDR, in hematopoietic cells and inhibitory effect of VEGF on apoptotic cell death caused by ionizing radiation. *Cancer Res.*, **55**, 5687–5692.
- KERR, J.F.R., WYLLIE, A.H. & CURRIE, A.R. (1972). Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br. J. Cancer*, **26**, 239–257.
- KIM, Y.M., BOMBECK, C.A. & BILLIAR, T.R. (1999). Nitric oxide as a bifunctional regulator of apoptosis. *Circ. Res.*, **84**, 253–256.
- KOCKX, M.M., DEMEYER, G.R.Y., MUHRING, J., BULT, H., BULTINCK, J. & HERMAN, A.G. (1996). Distribution of cell replication and apoptosis in atherosclerotic plaques of cholesterol-fed rabbits. *Atherosclerosis*, **120**, 115–124.
- KOCKX, M.M., DE MEYER, G.R.Y., MUHRING, J., JACOB, W., BULT, H. & HERMAN, A.G. (1998). Apoptosis and related proteins in different stages of human atherosclerotic plaques. *Circulation*, **97**, 2307–2315.
- KOYAMA, H., RAINES, E.W., BORNFELDT, K.E., ROBERTS, J.M. & ROSS, R. (1996). Fibrillar collagen inhibits arterial smooth muscle proliferation through regulation of Cdk2 inhibitors. *Cell*, **87**, 1069–1078.
- KROEMER, G. (1997). The proto-oncogene Bcl-2 and its role in regulating apoptosis. *Nat. Med.*, **3**, 614–620.
- KROEMER, G., ZAMZAMI, N. & SUSIN, S.A. (1997). Mitochondrial control of apoptosis. *Immunol. Today*, **18**, 44–51.
- KWAK, H.J., SO, J.N., LEE, S.J., KIM, I. & KOH, G.Y. (1999). Angiopoietin-1 is an apoptosis survival factor for endothelial cells. *FEBS Lett.*, **448**, 249–253.
- LANG, R.A. & BISHOP, J.M. (1993). Macrophages are required for cell death and tissue remodeling in the developing mouse eye. *Cell*, **74**, 453–462.
- LESZCZYNSKI, D., ZHAO, Y., LUOKKAMÄKI, M. & FOEGH, M.L. (1994). Apoptosis of vascular smooth muscle cells. Protein kinase C and oncoprotein Bcl-2 are involved in regulation of apoptosis in non-transformed rat vascular smooth muscle cells. *Am. J. Pathol.*, **145**, 1265–1270.
- LEVINE, J.S. & KOH, J.S. (1999). The role of apoptosis in autoimmunity: immunogen, antigen, and accelerant. *Semin. Nephrol.*, **19**, 34–47.
- LEVKAU, B., HERREN, B., KOYAMA, H., ROSS, R. & RAINES, E.W. (1998). Caspase-mediated cleavage of focal adhesion kinase p125FAK and disassembly of focal adhesions in human endothelial cell apoptosis. *J. Exp. Med.*, **187**, 579–586.
- LEVKAU, B., SCATENA, M., GIACHELLI, C.M., ROSS, R. & RAINES, E.W. (1999). Apoptosis overrides survival signals through a caspase-mediated dominant-negative NF- $\kappa$ B loop. *Nat. Cell. Biol.*, **1**, 227–233.
- LI, D., TOMSON, K., YANG, B., MEHTA, P., CROKER, B.P. & MEHTA, J.L. (1999a). Modulation of constitutive nitric oxide synthase, bcl-2 and Fas expression in cultured human coronary endothelial cells exposed to anoxia-reoxygenation and angiotensin II: role of AT1 receptor activation. *Cardiovasc. Res.*, **41**, 109–115.
- LI, D., YANG, B., PHILIPS, M.I. & MEHTA, J.L. (1999b). Proapoptotic effects of ANG II in human coronary artery endothelial cells: role of AT1 receptor and PKC activation. *Am. J. Physiol.*, **276**, H786–H792.
- LINDNER, H., HOLLER, E., ERTL, B., MÜLTHOFF, G., SCHREGLMANN, M., KLAUKE, I., SCHULTZ-HECTOR, S. & EISSNER, G. (1997). Peripheral blood mononuclear cells induce programmed cell death in human endothelial cells and may prevent repair: role of cytokines. *Blood*, **89**, 1931–1938.
- LIU, X., ZOU, H., SLAUGHTER, C. & WANG, X. (1997). DFF, a heterodimeric protein that functions downstream of caspase-3 to trigger DNA fragmentation during apoptosis. *Cell*, **89**, 175–184.
- LIZARD, G., DECKERT, V., DUBREZ, L., MOISANT, M., GAMBERT, P. & LAGROST, L. (1996). Induction of apoptosis in endothelial cells treated with cholesterol oxides. *Am. J. Pathol.*, **148**, 1625–1638.
- LUCAS, R., HOLMGREN, L., GARCIA, I., JIMENEZ, B., MANDRIOTA, S.J., BORLAT, F., SIM, B.K., WU, Z., GRAU, G.E., SHING, Y., SOFF, G.A., BOUCK, N. & PEPPER, M.S. (1998). Multiple forms of angiostatin induce apoptosis in endothelial cells. *Blood*, **92**, 4730–4741.
- MAJNO, G. & JORIS, I. (1995). Apoptosis, oncosis, and necrosis. *Am. J. Pathol.*, **146**, 3–15.
- MALLAT, Z., BENAMER, H., HUGEL, B., BENESSIANO, J., STEG, P.G., FREYSSINET, J.M. & TEDGUI, A. (2000). Elevated levels of shed membrane microparticles with procoagulant potential in the peripheral circulating blood of patients with acute coronary syndromes. *Circulation*, **101**, 841–843.
- MALLAT, Z., BESNARD, S., DURIEZ, M., DELEUZE, V., EMMANUEL, F., BUREAU, M.F., SOUBRIER, F., ESPOSITO, B., DUEZ, H., FIEVET, C., STAELS, B., DUVERGER, N., SCHERMAN, D. & TEDGUI, A. (1999c). Protective role of interleukin-10 in atherosclerosis. *Circ. Res.*, **85**, e17–e24.

- MALLAT, Z., HEYMES, C., OHAN, J., FAGGIN, E., LESÈCHE, G. & TEDGUI, A. (1999a). Expression of interleukin-10 in advanced human atherosclerotic plaques. Relation to inducible nitric oxide synthase expression and cell death. *Arterioscler. Thromb. Vasc. Biol.*, **19**, 611–616.
- MALLAT, Z., HUGEL, B., OHAN, J., LESÈCHE, G., FREYSSINET, J.M. & TEDGUI, A. (1999b). Shed membrane microparticles with procoagulant potential in human atherosclerotic plaques. A role for apoptosis in plaque thrombogenicity. *Circulation*, **99**, 348–353.
- MALLAT, Z., OHAN, J., LESÈCHE, G. & TEDGUI, A. (1997). Colocalization of CPP-32 with apoptotic cells in human atherosclerotic plaques. *Circulation*, **96**, 424–428.
- MARTIN, S.J., REUTELINGSPERGER, C.P.M., MCGAHON, A.J., RADER, J.A., VAN SCHIE, R.C.A., LAFACE, D.M. & GREEN, D.R. (1995). Early redistribution of plasma membrane phosphatidylserine is a general feature of apoptosis regardless of the initiating stimulus: inhibition by overexpression of Bcl-2 and Abl. *J. Exp. Med.*, **182**, 1545–1556.
- MASON, C.A., BIGRAS, J.L., O'BLENS, S.B., ZHOU, B., MCINTYRE, B., NAKAMURA, N., KANEDA, Y. & RABINOVITCH, M. (1999). Gene transfer in utero biologically engineers a patent ductus arteriosus in lambs by arresting fibronectin-dependent neointimal formation. *Nat. Med.*, **5**, 176–182.
- MEESON, A., PALMER, M., CALFON, M. & LANG, R. (1996). A relationship between apoptosis and flow during programmed capillary regression is revealed by vital analysis. *Development*, **122**, 3929–3938.
- MEREDITH, J., FAZELI, B. & SCHWARTZ, M. (1993). The extracellular matrix as a survival factor. *Mol. Biol. Cell*, **4**, 953–961.
- MEVORACH, D., MASCARENHAS, J.O., GERSHOV, D. & ELKON, K.B. (1998). Complement-dependent clearance of apoptotic cells by human macrophages. *J. Exp. Med.*, **188**, 2313–2320.
- MIOSSEC, C., DUTILLEUL, V., FASSY, F. & DIHERCEND, A. (1997). Evidence for CPP32 activation in the absence of apoptosis during T lymphocyte stimulation. *J. Biol. Chem.*, **272**, 13459–13462.
- MORISHITA, R., GIBBONS, G.H., ELLISON, K.E., LEE, W., ZHANG, L.N., YU, H., KANEDA, Y., OGIHARA, T. & DAZU, V.J. (1994). Evidence for direct local effect of angiotensin in vascular hypertrophy—in vivo gene transfer of angiotensin converting enzyme. *J. Clin. Invest.*, **94**, 978–984.
- MOULTON, K.S., HELLER, E., KONERDING, M.A., FLYNN, E., PALINSKI, W. & FOLKMAN, J. (1999). Angiogenesis inhibitors endostatin or TNP-470 reduce intimal neovascularization and plaque growth in apolipoprotein E-deficient mice. *Circulation*, **99**, 1726–1732.
- NICHOLSON, D.W., ALI, A., THORNBERRY, N.A., VAILLANCOURT, J.P., DING, C.K., GALLANT, M., GAREAU, Y., GRIFFIN, P.R., LABELLE, M., LAZEBNIK, Y.A., MUNDAY, N.A., RAJU, S.M., SMULSON, M.E., YAMIN, T.-T., YU, V.L. & MILLER, D.K. (1995). Identification and inhibition of the ICE/CED-3 protease necessary for mammalian apoptosis. *Nature*, **376**, 37–43.
- NISHIO, E. & WATANABE, Y. (1996). Oxysterols induced apoptosis in cultured smooth muscle cells through CPP32 protease activation and BCL-2 protein downregulation. *Biochem. Biophys. Res. Commun.*, **226**, 928–934.
- NOBLE, K.E., WICKREMASINGHE, R.G., DECORNET, C., PANAYIOTIDIS, P. & YONG, K.L. (1999). Monocytes stimulate expression of the Bcl-2 family member, A1, in endothelial cells and confer protection against apoptosis. *J. Immunol.*, **162**, 1376–1383.
- PARKES, J.L., CARDELL, R.R., HUBBARD, F.C.J., HUBBARD, D., MELTZER, A. & PENN, A. (1991). Cultured human atherosclerotic plaque smooth muscle cells retain transforming potential and display enhanced expression of the myc protooncogene. *Am. J. Pathol.*, **138**, 765–775.
- PERLMAN, H., MAILLARD, L., KRASINSKI, K. & WALSH, K. (1997). Evidence for the rapid onset of apoptosis in medial smooth muscle cells after balloon injury. *Circulation*, **95**, 981–987.
- POLLMAN, M.J., HALL, J.L. & GIBBONS, G.H. (1999b). Determinants of vascular smooth muscle cell apoptosis after balloon angioplasty injury: influence of redox state and cell phenotype. *Circ. Res.*, **84**, 113–121.
- POLLMAN, M.J., HALL, J.L., MANN, M.J., ZHANG, L. & GIBBONS, G.H. (1998). Inhibition of neointimal cell bcl-x expression induces apoptosis and regression of vascular disease. *Nat. Med.*, **4**, 222–227.
- POLLMAN, M.J., NAUMOVSKI, L. & GIBBONS, G.H. (1999a). Vascular cell apoptosis: cell type-specific modulation by transforming growth factor- $\beta$ 1 in endothelial cells versus smooth muscle cells. *Circulation*, **99**, 2019–2026.
- POLLMAN, M.J., YAMADA, T., HORIUCHI, M. & GIBBONS, G.H. (1996). Vasoactive substances regulate vascular smooth muscle cell apoptosis. Countervailing influences of nitric oxide and angiotensin II. *Circ. Res.*, **79**, 748–756.
- RE, F., ZANETTI, A., SIRONI, M., POLENTARUTTI, N., LANFRANCONE, L., DEJANA, E. & COLOTTA, F. (1994). Inhibition of anchorage-dependent cell spreading triggers apoptosis in cultured human endothelial cells. *J. Cell Biol.*, **127**, 537–546.
- RICHARDSON, B.C., LALWANI, N.D., JOHNSON, K.J. & MARKS, R.M. (1994). Fas ligation triggers apoptosis in macrophages but not endothelial cells. *Eur. J. Immunol.*, **24**, 2640–2645.
- ROSEN, A., CASCIOLA-ROSEN, L. & AHEARN, J. (1995). Novel packages of viral and self-antigens are generated during apoptosis. *J. Exp. Med.*, **181**, 1557–1561.
- ROSS, R. (1999). Atherosclerosis an inflammatory disease. *N. Engl. J. Med.*, **340**, 115–126.
- ROUIS, M., ADAMY, C., DUVERGER, N., LESNIK, P., HORELLOU, P., MOREAU, M., EMMANUEL, F., CAILLAUD, J.M., LAPLAUD, P.M., DACHET, C. & CHAPMAN, M.J. (1999). Adenovirus-mediated overexpression of tissue inhibitor of metalloproteinase-1 reduces atherosclerotic lesions in apolipoprotein E-deficient mice. *Circulation*, **100**, 533–540.
- RÜEGG, C., YILMAZ, A., BIELER, G., BAMAT, J., CHAUBERT, P. & LEJEUNE, F.J. (1998). Evidence for the involvement of endothelial cell integrin  $\alpha_v\beta_3$  in the disruption of the tumor vasculature induced by TNF and IFN- $\gamma$ . *Nat. Med.*, **4**, 408–413.
- SAKAHIRA, H., ENARI, M. & NAGATA, S. (1998). Cleavage of CAD inhibitor in CAD activation and DNA degradation during apoptosis. *Nature*, **391**, 96–99.
- SATA, M., PERLMAN, H., MURUVE, D.A., SILVER, M., IKEBE, M., LIBERMANN, T.A., OETTGEN, P. & WALSH, K. (1998). Fas ligand gene transfer to the vessel wall inhibits neointima formation and overrides the adenovirus-mediated T cell response. *Proc. Natl. Acad. Sci. U.S.A.*, **95**, 1213–1217.
- SATA, M. & WALSH, K. (1998). Oxidized LDL activates Fas-mediated endothelial cell apoptosis. *J. Clin. Invest.*, **102**, 1682–1689.
- SAVILL, J., FADOK, V., HENSON, P. & HASLETT, C. (1993). Phagocyte recognition of cells undergoing apoptosis. *Immunol. Today*, **14**, 131–136.
- SCATENA, M., ALMEIDA, M., CHAISSON, M.L., FAUSTO, N., NICOSIA, R.F. & GIACHELLI, C.M. (1998). NF-kappaB mediates alphavbeta3 integrin-induced endothelial cell survival. *J. Cell Biol.*, **141**, 1083–1093.
- SCHAUB, F.J.W., COATS, S.A., SEIFERT, R.A., RAMACHANDRAN, R.K., HAN, D.K.M. & SCHWARTZ, S.M. (1998). Regulated overexpression of the Fas-associated death domain (FADD) protein in seeded vascular smooth muscle cells causes apoptosis followed by recruitment of macrophages. *Circulation*, **98**, I-597. Abstract.
- SLOMP, J., GITTENBERGERDEGROOT, A.C., GLUKHOVA, M.A., VANMUNSTEREN, J.C., KOCKX, M.M., SCHWARTZ, S.M. & KOTELIANSKY, V.E. (1997). Differentiation, dedifferentiation, and apoptosis of smooth muscle cells during the development of the human ductus arteriosus. *Arterioscler. Thromb. Vasc. Biol.*, **17**, 1003–1009.
- SPEIR, E., MODALI, R., HUANG, E.S., LEON, M.B., SHAWL, F., FINKEL, T. & EPSTEIN, S.E. (1994). Potential role of human cytomegalovirus and p53 interaction in coronary restenosis. *Science*, **265**, 391–394.
- SPYRIDOPOULOS, I., SULLIVAN, A.B., KEARNEY, M., ISNER, J.M. & LOSORDO, D.W. (1997). Estrogen-receptor-mediated inhibition of human endothelial cell apoptosis—Estradiol as a survival factor. *Circulation*, **95**, 1505–1514.
- STEG, P.G., TAHLIL, O., AUBAILLY, N., CAILLAUD, J.M., DEDIEU, J.F., BERTHELOT, K., LE ROUX, A., FELDMAN, L., PERRICAUD, M., DENEFLÉ, P. & BRANELLEC, D. (1997). Reduction of restenosis after angioplasty in an atheromatous rabbit model by suicide gene therapy. *Circulation*, **96**, 408–411.
- STROMBLAD, S., BECKER, J.C., YEBRA, M., BROOKS, P.C. & CHERESH, D.A. (1996). Suppression of p53 and p21 expression by vascular cell integrin  $\alpha_v\beta_3$  during angiogenesis in vivo. *J. Clin. Invest.*, **98**, 426–433.
- TAN, E.M. (1994). Autoimmunity and apoptosis. *J. Exp. Med.*, **179**, 1083–1086.
- THOMAS, W.A., REINER, J.M., FLORENTIN, F.A., LEE, K.T. & LEE, W.M. (1976). Population dynamics of arterial smooth muscle cells. V. Cell proliferation and cell death during initial three months in atherosclerotic lesions induced in swine by hypercholesterolemic diet and intimal trauma. *Exp. Mol. Pathol.*, **24**, 360–374.

- THORNBERRY, N.A. & LAZEBNIK, Y. (1998). Caspases: enemies within. *Science*, **281**, 1312–1316.
- TOPOL, E.J., CALIFF, R.M., WEISMAN, H.F., ELLIS, S.G., TCHENG, J.E., WORLEY, S., IVANHOE, R., GEORGE, B.S., FINTEL, D., WESTON, M., *et al.* (1994). Randomized trial of coronary intervention with antibody against platelet IIb/IIIa integrin for reduction of clinical restenosis: results at six months. The EPIC Investigators. *Lancet*, **343**, 881–886.
- TRAN, J., RAK, J., SHEEHAN, C., SAIBIL, S.D., LACASSE, E., KORNELUK, R.G. & KERBEL, R.S. (1999). Marked induction of the IAP family antiapoptotic proteins survivin and XIAP by VEGF in vascular endothelial cells. *Biochem. Biophys. Res. Commun.*, **264**, 781–788.
- TRICOT, O., MALLAT, Z., HEYMES, C., LESÈCHE, G. & TEDGUI, A. (2000). Relation between endothelial cell apoptosis and blood flow direction in human atherosclerotic plaques. *Circulation*, in press.
- VAN ANTWERP, D.J., MARTIN, S.J., KAFRI, T., GREEN, D.R. & VERMA, I.M. (1996). Suppression of TNF- $\alpha$ -induced apoptosis by NF- $\kappa$ B. *Science*, **274**, 787–789.
- VIRCHOW, R. (1858). *Cellular Pathology as Based Upon Physiological and Pathological Histology*. p. 361. Birmingham, AL: Classics of Medicine Library.
- VON DER LEYEN, H., GIBBONS, G.H., MORISHITA, R., LEWIS, N.P., ZHANG, L., KANEDA, Y., COOKE, J.P. & DZAU, V.J. (1995). Gene therapy inhibiting neointimal vascular lesion: in vivo transfer of endothelial cell nitric oxide synthase gene. *Proc. Natl. Acad. Sci. U.S.A.*, **92**, 1137–1141.
- WANG, B.Y., HO, H.K., LIN, P.S., SCHWARZACHER, S.P., POLLMAN, M.J., GIBBONS, G.H., TSAO, P.S. & COOKE, J.P. (1999). Regression of atherosclerosis: role of nitric oxide and apoptosis. *Circulation*, **99**, 1236–1241.
- WATANABE, Y. & DVORAK, H.F. (1997). Vascular permeability factor/vascular endothelial growth factor inhibits anchorage-disruption-induced apoptosis in microvessel endothelial cells by inducing scaffold formation. *Exp. Cell. Res.*, **233**, 340–349.
- YACHIE, A., NIIDA, Y., WADA, T., IGARASHI, N., KANEDA, H., TOMA, T., OHTA, K., KASAHARA, Y. & KOIZUMI, S. (1999). Oxidative stress causes enhanced endothelial cell injury in human heme oxygenase-1 deficiency. *J. Clin. Invest.*, **103**, 129–135.
- YAMADA, T., AKISHITA, M., POLLMAN, M.J., GIBBONS, G.H., DZAU, V.J. & HORIUCHI, M. (1998). Angiotensin II type 2 receptor mediates vascular smooth muscle cell apoptosis and antagonizes angiotensin II type 1 receptor action: an in vitro gene transfer study. *Life Sci.*, **63**, L289–L295.
- YUSUF, S., SLEIGHT, P., POGUE, J., BOSCH, J., DAVIES, R. & DAGENAIS, G. The Heart Outcomes Prevention Evaluation Study Investigators. (2000). Effects of an angiotensin-converting-enzyme inhibitor, ramipril, on death from cardiovascular causes, myocardial infarction, and stroke in high-risk patients. *N. Engl. J. Med.*, **342**, 145–153. Published erratum appears in *N. Engl. J. Med.* 2000; 342: 748.
- ZHENG, B. & CLEMMONS, D.R. (1998). Blocking ligand occupancy of the  $\alpha_v\beta_3$  integrin inhibits insulin-like growth factor I signaling in vascular smooth muscle cells. *Proc. Natl. Acad. Sci. U.S.A.*, **95**, 11217–11222.

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